EFFECT OF SLEEP DEPRIVATION ON VISUAL AND AUDITORY EVOKED POTENTIALS IN FEMALE NIGHT SHIFT PERSONNEL OF A TERTIARY CARE HOSPITAL

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the requirements for the award of the degree of

M.D. PHYSIOLOGY

Branch V

April 2017

EFFECT OF SLEEP DEPRIVATION ON VISUAL AND AUDITORY EVOKED POTENTIALS IN FEMALE NIGHT SHIFT PERSONNEL OF A TERTIARY CARE HOSPITAL

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the requirements for the award of the degree of

M.D. PHYSIOLOGY

Branch V

April 2017

CERTIFICATE

This is to certify that this dissertation entitled "**EFFECT OF SLEEP DEPRIVATION ON VISUAL AND AUDITORY EVOKED POTENTIALS IN FEMALE NIGHT SHIFT PERSONNEL OF A TERTIARY CARE HOSPITAL "** is a bonafide record of the work done by **Dr. Archana Chandran** under my guidance and supervision in the Department of Physiology during the period of her postgraduate study for **M.D. Physiology [Branch – V]** from 2014-2017.

Dr. P.S.Krishnamurthy, M.D.,
[Guide]
Professor and Head,
Department of Physiology,
Sree Mookambika Institute of Medical Sciences, Kulasekharam,
Kanyakumari District,
Tamil Nadu – 629 161.

Dr. Rajagopalan Asari, M.D.,
[Co-Guide]
Associate Professor,
Department of Physiology,
Sree Mookambika Institute of Medical Sciences, Kulasekharam,
Kanyakumari District,
Tamil Nadu – 629 161.

Dr. Rema. V. Nair, M.D., D.G.O.,
[Director]
Sree Mookambika Institute of Medical Sciences, Kulasekharam,
Kanyakumari District,
Tamil Nadu – 629 161.

DECLARATION

I, Dr. Archana Chandran here by submit the dissertation titled **"Effect of Sleep Deprivation on Visual and Auditory Evoked Potentials in Female Night Shift Personnel of a Tertiary Care Hospital"** done in partial fulfillment for the award of the degree M.D. Physiology [Branch – V] in Sree Mookambika Institute of Medical Sciences, Kulasekharam. This is an original work done by me under the guidance and supervision of **Dr. P.S.Krishnamurthy.**

Dr. P.S.Krishnamurthy, M.D.,
[Guide]
Professor and Head,
Department of Physiology,
Sree Mookambika Institute of Medical Sciences, Kulasekharam,
Kanyakumari District,
Tamil Nadu – 629 161.

Dr. Archana Chandran, M.B.B.S.,
Postgraduate Student
Department of Physiology,
Sree Mookambika Institute of Medical Sciences, Kulasekharam,
Kanyakumari District,
Tamil Nadu – 629 161.

EFFECT OF SLEEP DEPRIVATION ON VISUAL AND AUDITORY EVOKED POTENTIALS IN FEMALE NIGHT SHIFT PERSONNEL OF A TERTIARY CARE HOSPITAL

Dissertation

Submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the requirements for the award of the degree of

M.D. PHYSIOLOGY

Branch V

ACKNOWLEDGEMENT

I would like to express my gratitude to my professor and Head of Department of Physiology, my mentor and guide **Dr. P.S Krishnamurthy,** for his valuable and constant guidance, supervision and support to endure the hardship throughout the study. His patience and understanding during the times of difficulties in the study period helped me a lot to overcome the circumstances. His constant motivation has helped me to overcome all the challenges and difficulties that I came across during this research work. His encouragement from the initiation of this research to its culmination has always been profound. It has been an extraordinary experience working under him.

I am very much grateful and thankful to my Co-Guide **Dr. Rajagopalan Asari,** Associate Professor, Department of Physiology for his valuable support and guidance all throughout the study. He had always been a source of inspiration and had been of immense help with his extensive ideas, valuable contributions, patience and generous encouragement during the research work and preparation of the manuscript.

I extend my thanks to my Associate Professor **Dr. P. Prabhakar** for his valuable suggestion, support and encouragement throughout the study. I am also thankful to the Assistant professors **Dr. Rajesa Nandini , Dr. D. S .Florence Nesa Bella, Dr.Benjamin Jebaraj D and Dr Lisha Vincent** for their valuable inputs during all the stages of my study.

I extend my sincere heartfelt thanks to **Dr. Velayuthan Nair,** Chairman and **Dr. Rema V. Nair,** Director, for permitting me to carry out the study in the

hospital and providing facilities to accomplish my dissertation work. Then I would also like to thank the Principal of the Institution **Dr. Padmakumar** for his valuable support extended to me.

I express my special thanks to my colleague **Dr. Jiya Michael ,** for giving me uncountable constructive ideas and encouragement to do the study. I also thank my senior postgraduate **Dr. Hoshea Jeba Ruth S** for her help and support.

I thank my husband, **Dr. Arjun.G.Nair** for the technical assistance and help that he has extended to me during the study period.

I am thankful to all other nonteaching staff members for their support and cooperation in completing this work.

Table of Contents

INTRODUCTION

1. Introduction

Healthy sleep requires adequate duration, good quality, appropriate timing and regularity, and the absence of sleep disturbances or disorders. Sleep is essential for optimal health. Consistency is the cornerstone of a healthy sleep routine. A consistent routine can make both falling asleep and getting up in the morning easier, less stressful, and more relaxing. The American Academy of Sleep Medicine recommends eight hours of sleep per night on a regular basis to promote optimal health among adults aged 18 to 60 years. A normal sleep-wakeful cycle maintains the synchronization between the external environment and internal biological clock. The duration of normal sleep changes when a person is engaged in shift work especially night shift.[1]

Shift work is unavoidable because of continuous availability of essential services like medical, police, military, transport, electricity etc. Night workers are required to stay awake when their internal clock are preparing them for sleep and to sleep when preparing for wakefulness. So their circadian rhythm which is keyed to daylight and darkness is disrupted. As a result, it leads to substantial deterioration in sleep quality and duration.[2] The most immediate consequence of shift work is impaired alertness, which has widespread effects on core brain functions — reaction time, decision making, information processing and the ability to maintain attention. Alternations in the sleep pattern is associated with sleep disorders, stress, anxiety, depression, peptic ulcer disease, hypertension, diabetes, coronary heart disease, miscarriage, low-birth weight, preterm birth, increased risk of endometrial cancer and a variety of

neurocognitive deficits. The health and economic costs of shift work-related sleep–wake disturbances are high, taking into account the combined effects of impaired sleep, workplace and road accidents, mood disorders, lost productivity and cardiovascular health. Thus, changes in the circadian rhythm can lead to deleterious effects on normal neurophysiological functioning. [3-6]

Evoked potentials are electrical activities that occur in the neural pathways in response to a variety of stimuli. Understanding evoked potentials bears importance in terms of controlling the entire pathway from stimulation point to the cortical areas, in other words, to the primary cortex. By examining evoked potentials, one can find answers to many questions such as whether the response against the stimulus reach intended destinations on time, the response show any loss of intensity, any problem in the neural pathways and its exact location.[7] Almost any sensory modality can be tested in clinical practice. The most often encountered evoked potentials are the visual evoked potential (VEP) and brainstem auditory evoked potential (BAEP). Visual evoked potential (VEP) is the electrical potential difference recorded from the scalp in response to visual stimuli. VEP provide a qualitative and quantitative measurement of visual pathway.[8] Brainstem auditory evoked potential (BAEP) are potentials recorded from the ear and vertex in response to brief click stimulus. BAEP provide a qualitative and quantitative measurement of auditory pathway. So, evoked potential recording methods are non-invasive, highly objective and informative clinical tool to assess neurophysiological functioning.[9]

There are very few studies showing effects of altered circadian rhythm on vision and hearing. Certain abnormalities in the evoked potentials reflect subclinical involvement of Central Nervous System even before the disease clinically manifests.[10,11] It is important to know the effects of sleep deprivation in night shift workers in order to understand the physiological actions and underlying mechanism of disrupted circadian rhythm on visual and hearing functions. The present study is aimed to explore the changes in the VEP and BAEP waves in night shift personnel compared to day shift.

HYPOTHESIS

&

JUSTIFICATION

2.1 Hypothesis

Significant changes in visual and auditory evoked potentials in female night shift personnel compared to day shift.

2.2 Justification

The circadian rhythm is a cycle of biochemical, physiological and behavioural processes co-ordinated by the suprachiasmatic nucleus (SCN) of the anterior hypothalamus.[9,12] Recently discovered intrinsically photosensitive melanopsin retinal ganglion cells contribute to the maintenance of pupil diameter. They also provide the primary environmental light input to the SCN for photoentrainment of the circadian rhythm.[13] Rotating shift workers work during the night and their sleep pattern gets altered which in turn disrupts the circadian rhythm. The disrupted circadian rhythm brings about change in the pupil size. This circadian variation in pupil size might alter the area of retinal illumination. This may affect the visual evoked potential.[9,11] Hence, this study is aimed at finding whether there is any change in visual evoked potential due to altered circadian rhythm caused by sleep deprivation in night shift workers of a tertiary care hospital.

There are studies showing increased Visual Reaction Time and Auditory Reaction Time in response to acute sleep deprivation in the night shift workers.[14] The behavioral response in these studies is the summation of a number of processes—from the initial encoding of information and motor execution of the response to the later cognitive processes, such as

attention and memory. So, the behavioral response in these studies suggest that some processes are affected by sleep deprivation. These behavioral studies may not be sensitive enough to detect abnormalities in the under lying cognitive processes. One way to examine these underlying neural processes is by using visual and auditory evoked potentials, a scalp-derived measure of neural activity. Examination of the amplitude and latency of the responses may help to determine where in the sequence of information processing sleep deprivation is influencing these behavioral response.[15]

There are very limited number of studies showing effects of disrupted circadian rhythm on vision and hearing. Changes in the evoked potentials reflect subclinical involvement of Central Nervous System even before the disease clinically manifests.[9-11] So it is important to know the effects of sleep deprivation in night shift workers in order to understand the physiological actions and underlying mechanism of disrupted circadian rhythm on visual and hearing functions.

AIMS AND OBJECTIVES

2. Aims and Objectives:

1. To compare the Visual evoked potential between day shift and night shift personnel working in a tertiary care hospital.
2. To compare the Brainstem auditory evoked potential between day shift and night shift personnel working in a tertiary care hospital.

REVIEW OF LITERATURE

4. Review of Literature

4.1 Sleep

Sleep is a periodic reversible physiological state of loss of consciousness, which can be dispelled by adequate stimulation and which is necessary for the recoupment and well being of individuals.[16]

The sleep is the natural periodic state of rest for mind and body with closed eyes characterized by partial or complete loss of consciousness. Loss of consciousness leads to decreased response to external stimuli and decreased body movements. Depth of sleep is not constant throughout the sleeping period. An individual is kept awake by activation of reticular activating system. Sleep occurs due to periodic shut down of reticular activating system by influences from brainstem and other regions of brain. Kleitman and coworkers[17] through EEG and polygraphic analysis, described that normal sleep consists of recurring cycles of different stages, each of which is characterized by specific EEG changes associated with autonomic and endocrine changes. The physiological criteria are based on the findings of electro-encephalography (EEG), electrooculography (EOG) and electromyography (EMG).

4.1.1 History

The electrical activity in brain during non-REM sleep was first discovered using electroencephalograph (EEG) in 1937.In the middle of the 19th century, REM sleep was observed and its relation with dreams

was explained by Kleitman and his colleagues. Since the advent of REM sleep, physiology of sleep was no longer a mystery and lot of theories regarding sleep followed. After 7 years of REM sleep discovery, Kleitman and Dement proposed the stages of sleep. In the later part of 19th century, sleep was defined as an essential global biological phenomenon that influences the activity of most organ systems in the human body. Knowledge of the functioning of sleep was extended by the contributions from various disciples of medical fields like neurology, respiratory medicine, psychiatry, otorhinolaryngology, anatomy, pharmacology and physiology.[16]

During 1980s, Rechtschaffen et al.[18] did experiments with rats proving sleep is essential for wellbeing. The animals were subjected to total sleep deprivation and were observed for days. They developed skin diseases, observed paradoxical loss of bodyweight in spite of polyphagia, contracted pyogenic infections, and died within a month. Since then, the etiopathogenesis of sleep related disorders were intensively researched for better understanding of the problems.[16,17]

4.1.2 Types of sleep

Based on the three physiological measurements(EEG, EOG and EMG), sleep is divided into two states with independent functions and controls: (a) non rapid eye movement (NREM) and (b) rapid eye movement (REM) sleep, both alternating in a cyclic manner.[19]

4.1.2.1 NREM Sleep

Non - rapid eye movement (NREM) sleep is also known as slow wave sleep (SWS),because in this type of sleep brain waves are very slow. In normal adults, sleep mostly begins with NREM sleep. It is a rest type of sleep which a person experiences during the first hour of sleep after having been awake for many hours. The sleep cycle begins with NREM sleep. There are four stages of slow wave sleep, person when falls asleep passes sequentially through these four stages of increasingly deep sleep. After that sleep lightens and he enters into REM period. Mean duration of sleep cycle is 1 hour 20 minutes of which NREM sleep constitutes about three-fourths of mean sleep duration and REM sleep about one-fourth.[19]

4.1.2.1. A. Stages

The NREM sleep progresses in an orderly fashion from light to deep sleep through four stages as:

Stage 1 of NREM sleep (stage of very light sleep)shows low amplitude mixed frequency activity in electroencephalogram (EEG). However, the mild to moderate stimuli are often unable to produce a full arousal. This stage constitutes only three percent of total sleep and normally lasts approximately five minutes of sleep cycle. Sensory perception is intact during this stage and can be impeded by stimulus like disrupting sounds. EEG recordings show regular alpha (α) waves of reduced voltage with low frequency roughly around 10 cycles per second.[17-20]

Stage 2 of NREM Sleep (stage of light sleep) is characterized by the appearance of sleep spindles. Sleep spindles are bursts of alpha-like waves of frequency between 10-14 Hz and voltage of 50micro volt. Auditory stimuli during this phase readily evoke the k-complexes in the EEG. The k-complex consists of one or two high voltage waves followed by a brief 14Hz activity.[17-20] Gais et al.[21] proposed the role of sleep spindles in memory integration. He found out that the density of the sleep spindles were more in subjects who learn a new assignment than the control group.

Stage 3 of NREM sleep (stage of moderate sleep) is characterized by an EEG that display low frequency (0.5-2 Hz) high amplitude waves. [17-20]

Stage 4 of NREM sleep (stage of deep sleep) produces EEG pattern of dome like with low frequency (0.5-4 Hz) and high amplitude (20-200 micro volt) waves called delta (δ) waves. It is very difficult to wake up the subject in this stage. The maximum time of sleep is spent at stage 4 in young adults. [17-20]

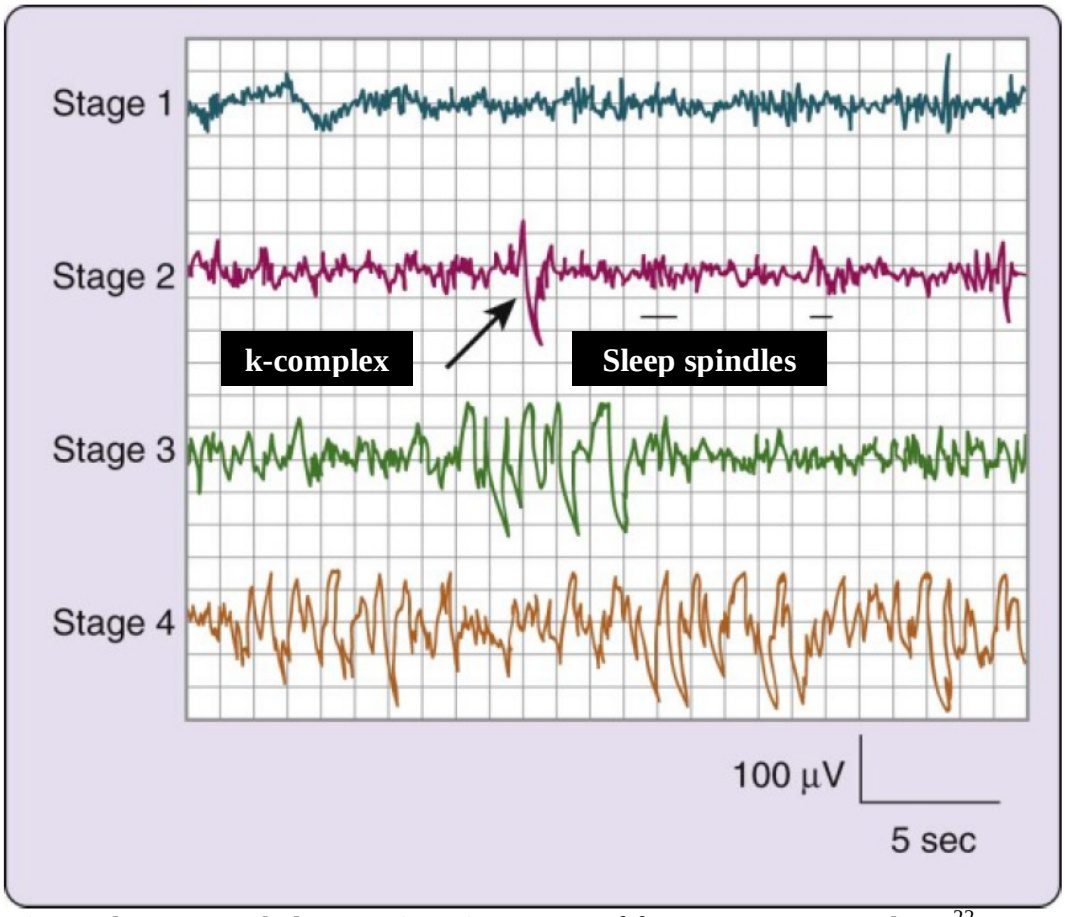


Fig 1: Electroencephalogram (EEG) patterns of four stages NREMsleep.[22]

4.1.2.1. B. Physiological changes during non-REM Sleep [23]

The physiological events that happen during non-REM sleep are well indulged in healthy individuals, although compromised in those having derangement of body systems like cardiovascular diseases. Physiological changes occurring are as follows:-

- **Brain Activity:** The brain activity decreases from wakefulness.
- **Heart and blood vessels:** During sleep, the heart rate lowers to about 40-60 beats per minute and systolic blood pressure falls to about 90 to 110mm Hg. Lowest level is reached about fourth hour of sleep and remains at this level till a short time before waking up. Floras and his co-

workers[24] found out that there is an increased propensity to cause myocardial infarction in the morning owing to increased heart rate and blood pressure that follows awakening.

- **Sympathetic system:** There is a reciprocal relationship between sympathetic activity and non-REM sleep. Nevertheless, there is a hike in sympathetic activity during non-REM sleep due to the short elevation in blood pressure and heart rate that trails the k-complexes.
- **Respiratory system:** The ventilation and respiratory flow decreases during sleep as airway resistance increases. During non-REM sleep, there is suppression of hypoxic respiratory drive and cough reflex. The rate as well as the force of respiration is decreased. Respiration becomes irregular and Cheyne-stokes type of periodic breathing may develop.
- **Cerebral blood flow:** The overall blood flow to the brain and metabolic processes are greatly decreased during non-REM sleep.
- **Kidney:** During non-REM sleep there is decreased glomerular filtration of electrolytes and may lead to increased levels of sodium, potassium and chloride in blood.
- **Hypothalamus:** Non-REM sleep resets the thermostat to a lesser set point than it is when awake. This may contribute to shivering during sleep.
- **Hormones:** Non-REM sleep can exert its influence on the secretion as well as release of certain hormones involved in the circadian rhythm like somatotropin, thyroxine, and melatonin. Normally, the secretion of

somatotropin occurs after a couple of hours of commencement of sleep during non-REM sleep.

- **Eye:** Eyes begin slow rolling movement until they finally stop in stage 4 with eyes turned upwards.

4.1.2.1. C. Behavioral changes during non-REM Sleep [23,25]

Behaviorally the NREM sleep is characterized by progressive reduction in consciousness and an increasing resistance to being awakened.

4.1.2.2 REM Sleep

Rapid eye movement (REM sleep) is also called "fast wave sleep" or "desynchronized sleep", or 'paradoxical sleep' or 'dream sleep' or 'deepest sleep'. In adults, the REM sleep follows NREM sleep, while in adults entry into sleep occurs through REM sleep.[26]

Another characteristic feature of REM sleep is the appearance of ponto-geniculo-occipital spikes (PGO spikes) in EEG. PGO spikes are phasic potentials that occur in groups of three to five. They originate in pons and through lateral geniculate body travel to occipital cortex.[23,26]

Bader et al.[27] in 2003 explained the principal role in loss of muscle tone and reflexes in REM sleep for the reason that it stops the person from “acting out” their dreams or nightmares while taking a nap.

Dement et al.[28] in 1957 found that approximately eighty percentage of individual waking up during the rapid eye movement sleep results in realistic remembrance of dream.

4.1.2.2. A. Physiological changes during REM sleep[20-22]

Rapid eye movements are the hallmark of this state of sleep. These are characterized by bursts of small jerky movements that bring the eye from one fixation point to another to allow a sweeping of visual images of dreams.

- **Brain activity:** Sensory and motor cortical activity is increased.
- **Heart and Blood vessels:** Slight elevation in number of heart beats and blood pressure.
- **Sympathetic activity:** Sympathetic activity increases significantly from wakefulness.
- **Cerebral blood flow:** The overall blood flow to the brain and metabolic processes are greatly increased during NREM sleep. Madsen et al.[29] recorded blood flow is increased in the limbic system which is concerned with change in emotions and Broadmann's area 4 while sleeping.
- **Respiratory system:** Parker and his co-workers[30] in 2005 proposed that the ventilation and respiratory flow decreases during REM sleep as upper airway resistance increases. The rise in airway resistance is due to lack of respiratory muscle tone. The rate as well as the force of respiration is decreased during REM sleep.

- **Muscle tone**: Muscle tone is reduced due to inhibition of spinal motor neurons via brainstem mechanisms. Snoring during sleep results from partial obstruction of airways caused by relaxed tongue in supine position. Twitching of limb musculature occurs occasionally. Bader et al.[27] explained that this muscle twitching do not produce injuries or awaken the individual since muscle tone is reduced tremendously during REM sleep.
- **Sexual arousal:** Penile erection in males and engorgement of clitoris in females may occur during REM sleep.
- **Temperature**: Non-REM sleep resets the thermostat to a lesser set point than it is when awake. This may contribute to shivering during NREM sleep and it come to an end in REM Sleep.

4.1.2.2. B. Behavioral changes

It is difficult to arouse an individual from REM Sleep as it is from deep sleep. However when awakened from REM sleep, the individual is immediately alert and aware of the environment. Dreaming occurs during REM sleep, so it is also called dream sleep. There is 80%of arousals from REM sleep is due to vivid dream recall.[31]

4.1.3 Theories of sleep

- **Cerebral anemia theory**

It was considered that reduction of blood flow to the brain due to fatigue of the vasomotor centre caused sleep. But this theory was given up when it was found by actual measurements that the blood flow to the brain was not reduced during sleep. However, advanced techniques now

available for measuring regional blood flow in brain show that blood flow to some parts of the brain may be reduced, even though the total blood flow remains unchanged.[16,17,31]

- **Fatigue theory**

  Fatigue of the neuromuscular mechanism concerned in the maintenance of muscle tone was responsible for inducing sleep. However, this theory is no longer accepted. Also while the fatigue that occurs after heavy muscular work may cause sleep it does not always do so. This plausible simplistic theory however does not have wide acceptance. [16,17,31]

- **Chemical theories**

  It was found that electrical stimulation of median raphe nuclei located in the lower pons and medulla induces sleep by releasing serotonin. It was considered that serotonin was sleep inducing. But this could not be reconciled with the finding that blood levels of serotonin were lower during sleep than during wakefulness. Some studies indicate that serotonin is the most likely neurotransmitter involved in sleep, its exact role in the sleep mechanism is not clear. [16,17,31]

  It has also been proposed that prostaglandins may also have a role in sleep mechanism. Prostaglandin-D_2[PGD_2] which is released in the medial pre-optic region of the hypothalamus increases slow wave sleep and REM sleep, but release of prostaglandin-E_2[PGE_2] causes wakefulness.[31]

It was also been confirmed that prolonged wakefulness causes the accumulation in the cerebrospinal fluid of peptides which rapidly induces sleep in a waking animal. It appears to be a low molecular weight peptide produced in the brain, but the exact nature of this "sleep peptide" has not been determined. A lipid is also said to be produced in the brain, which may induce sleep. [16,17,31]

- **Pavlov's theory of conditioned inhibition**

  Pavlov found during his experiments on conditioned reflexes in animals, that inhibition of conditioned reflexes was accompanied by drowsiness or sleep. He proposed that sleep was induced by spread of conditioned inhibition over the entire cerebral cortex. But sleep is not entirely a cortical phenomenon. [16,17,31]

- **Diencephalon and other neuronal centers**

  Rudolf Hess postulated the existence of a sleep centre in the anterior hypothalamus and a waking centre in posterior hypothalamus. However there are a number of areas, stimulation of which causes sleep.

(a) In the anterior hypothalamus in the region of the preoptic nucleus is the basal forebrain sleep zone. Both high and low frequency stimulation of this part induces sleep. The suprachiasmatic portion is also sleep inducing.

(b) Low frequency electrical stimulation of the posterior hypothalamus and the nearby intralaminar nuclei causes sleep. This is referred to as the diencephalic sleep zone.

(c) In the reticular formation of the medulla near the nucleus of the tractus solitarius,is the medullary synchronizing zone. Low frequency electrical stimulation causes sleep. The effect is probably by activation of the neurons in the raphe nuclei.

(d) Stimulation of midline raphe nuclei in the lower pons and medulla induces sleep, fibers from these nuclei connect with the hypothalamus and spread to the cortex via the thalamus. [16,17,31]

- **Kleitman's theory of diminished sensory input**

Kleitman postulated that inactivity of the cerebral cortex resulting from reduction in the number of sensory afferent and proprioceptor impulses was responsible for sleep. This is supported by the fact that cutting off or reducing visual and auditory stimuli (darkness, silence) of touch pressure and temperature stimuli and of proprioceptor stimuli, favors sleep. Lesion of the sleep promoting zones cause a high state of wakefulness. e,g, raphe nuclei, medullary synchronizing zone and parts of hypothalamus; the effect is due to release of the excitatory reticular regions of upper pons and midbrain from inhibitory effects. [16,17,31]

- **Role of Reticular Formation**

In 1940, Magoun et.al. discovered the role of the brainstem reticular formation and its ascending projections to the cortex in maintenance of cortical excitability and consciousness. When the number of impulses transmitted from the reticular formation to the cortex was increased, wakefulness and alertness occurs. The neurotransmitters

involved are noradrenaline and probably serotonin released in the neurons of the locus ceruleus, midbrain raphe, when the number of impulses transmitted is reduced sleep occurs. [32]

- **Aminergic theory of sleep**

The neurohumoral theory of sleep was put forward by Jouvet suggested that noradrenaline and serotonin are involved in sleep-wakefulness. Fernandez-Peon also has noted acetylcholine, as the neurotransmitter balancing the actions of nor-epinephrine and serotonin in the sleep-waking rhythm.[32]

4.1.4 Sleep wake cycle

Sleep refers to a state of unconsciousness from which the individual can be aroused by sensory or other stimuli. When asleep, an individual is not aware of the environment and is unable to perform activities that require consciousness. The passage from waking state to sleep is usually gradual. There is drowsiness, increasing sensation of fatigue, indifference to environmental stimuli, reduction in motor activity and diminished muscle tone. The eyelid droop over the eyes and there may be slow side to side eye movements. The onset is favored by suppression of stimuli and facilitation of muscular relaxation. Silence, darkness, optimal temperature, posture and mental composure favors onset of sleep. The onset is retarded by strong stimuli(especially loud sounds, bright light), motor activity, mental excitement and worry. Sometimes the onset may be less gradual and more abrupt. During sleep,

the stimulus pulse transfer becomes less frequent between the reticular formation and cerebral cortex.[33]

Human sleep wake cycle is the most substantially explored biological rhythm. Existing studies have proved that sleep functions in restoration of physiological and biological process, learning and memory consolidation and conservation of energy. The cycle is modulated by the two processes: (i) circadian rhythm (ii) homeostasis. The circadian rhythm modulates by helping in initiation of sleep during night and by preventing sleep during day time. So this process is entirely dependent on time. In contrast, the process of homeostasis regulates the pattern of sleep based on the duration of previous wake and sleep states. After waking, there is decreased tendency to sleep, but it gradually increases and finally results in sleep. Similarly, during sleep the homeostatic processes slowly decrease the tendency to sleep till the subject wakes up. So, both the process of circadian rhythm and homeostasis functions parallel to regulate the initiation, cessation and maintenance of sleep and wakeful states.[33,34]

Sleep and wakefulness like many of the body's regulatory mechanisms, have circadian rhythm of about 24 hrs. A newborn infant has many cycles of sleep and wakefulness in 24hrs, but after the age of 2 years a single sleep-wake cycle consists of 7-8hrs of sleep and 16-17hrs of wakefulness.[34]

Sleep-wake cycle, like other circadian rhythms, is endogenous. The biological clock controlling the circadian rhythm is SCN of the anterior hypothalamus. The presence of environmental cues is not necessary for the functioning of circadian rhythm. But the rhythms can be modulated and can adapt themselves to the Zeitgebers or time givers of the environmental cues. For example, sunlight is a very potent timing cue. The retino-hypothalamic tract helps in photoentrainment of the circadian rhythm.[33-35]

4.1.4.1 Factors influencing Sleep-Wake Cycle [36]

Sleep is, in essence, food for the brain, and insufficient sleep can be harmful, even life-threatening. When hungry for sleep, the brain becomes relentless in its quest to satisfy its need and will cause feelings of "sleepiness," decreased levels of alertness or concentration, and, in many cases, unanticipated sleep. Numerous factors determine the quality and quantity of sleep. Quality of sleep is defined as the subject's ability to maintain sleep and to get adequate amount of both REM and non-REM sleep whereas, quantity of sleep is the total duration the subject spends in each stage of sleep.

- **Age**: Sleep pattern differs with age. Premature babies spent only 20% of the time in NREM sleep in contrast to mature babies who have equal distribution of both REM and NREM sleep. Duration of NREM sleep decreases so drastically above 60 years that the stage no longer exists.

- **Interest**: Motivation to override sleep and to be wakeful. Example, even if a subject is exhausted he might be wakeful if the movie or concert is entertaining. This is experienced vice-versa also, that is there is minimum interest to be awake resulting in sleep.
- **Customs and Beliefs:** Differs from persons to persons. Rest and sleep can be affected by certain customs and beliefs that the individual holds. For example: Chinese use of medicated tea as an alternative to sedatives to promote relaxation and sleep..
- **Lifestyle:** Sleep pattern can be influence by the lifestyle and habits the individual possesses.
    i. **Physical exertion**: Only physical activity within two-three hours before going to sleep will inversely affect the quality of sleep. Otherwise physical exertion increase fatigue and tiredness and induces relaxation resulting in sleep. So it is advised that moderate exercise can increase the quality of sleep.
    ii. **Dietary Habits**: Protein-rich foods contain tryptophan and can decrease sleep whereas carbohydrate-rich foods contain serotonin which can enhance sleep.
    iii. **Alcoholic and non-alcoholic beverages**: Mild to moderate consumption of alcohol containing beverages favors sleep but large amount can adversely affect sleep especially REM sleep and may contribute to the hangover experience the following day. Non-

alcoholic beverages like coffee, tea, cola-drinks contains caffeine that can act as a CNS stimulant and helps to counteract drowsiness.

iv. **Tobacco**: Tobacco contains nicotine that has excitatory effects on the body. Smokers and tobacco-chewers mostly experience difficulty in initiating and maintaining sleep, so are considered as "light sleepers". Individuals who have quit themselves from usage of nicotine claimed better quality of sleep than before.

- **Environment**: Environmental changes can alter the sleep pattern by either promoting or hindering sleep. Most people sleep best in their usual home environment. Sleeping in a strange or new environment can tends to influence both REM and NREM. Light is one of the most important external factors that can affect sleep. It does so both directly, by making it difficult for people to fall asleep, and indirectly, by influencing the timing of our internal clock and thereby affecting our preferred time to sleep.
- **Shift work and Jet lag :** Asynchrony between light exposure and internal clock arises due to shift work or jet-lag. Light exposure sets our internal clock to the appropriate time. This is known as photoentrainment of circadian rhythm. However, the synchrony between light exposure and internal clock is disrupted in night shift workers and in people travelling across different time zones. This asynchrony can result in either in insomnia or excessive sleepiness. These symptoms are usually experienced in physicians, nurses, police, pilots and public safety workers.

- **Drugs**: Many prescription medications like adrenergic blockers and over-the-counter drugs like cold remedies can affect both quality and quantity of sleep.
- **Psychological Stress**: Sleep is disturbed by life situations and illnesses. A stressed person gets inadequate sleep and lack of sleep can add on to more stress and anxiety leading to a vicious cycle.

4.1.4.2 Physiological significance [37]

Sleep is an indispensable phenomenon. Its physiological significance is highlighted.

a) Sleep may serve as a period of body's rest and metabolic restoration as evidenced by following physiological changes during non-REM Sleep.
    - Pulsatile release of growth hormone and gonadotropins from the pituitary
    - Decrease in blood pressure, heart rate and respiration.

b) Sleep is necessary for certain forms of learning

c) In experimental animals, learning sessions do not improve performance until a period of SWS or SWS plus REM sleep has occurred. However, it is not known why sleep is necessary and there is as yet no clinical correlate to this experimental observation.

d) REM sleep is necessary for mental well being.

e) The correlation between dreaming and REM sleep indicates that the brain is highly active at this time. This may allow for the expression, through dreams, of concern in the subconscious and for long term

chemical and structural changes that brain must undergo to make learning and memory possible.

f) REM sleep plays an important role in homeostatic mechanism.

g) It is evident from the observation that when the experimental animals are completely deprived of REM sleep for long periods, they lose weight in spite of increased caloric intake and finally die.

4.1.4.3 Regulation [35-37]

The processes underlying sleep regulation are consisting of (a) a homeostatic process responsible for the rise of sleep propensity during waking and its dissipation during sleep;(b) a circadian process that is basically independent of prior sleep and waking, and is responsible for the alternation of periods with high and low sleep propensity; and (c) a circadian process occurring within the sleep episode and representing the alternation of the two basic sleep states NREM sleep and REM sleep**.**

The three vigilance states (wakefulness [W], non-rapid eye movement sleep [NREM], rapid eye movement sleep [REM]) are controlled by distinct, but interconnected, networks of neurons. The sleep or arousal network consists of separate systems of wakefulness (W)-promoting and NREM-promoting neurons, located in nuclei in the basal forebrain, diencephalon and brainstem. Each neuronal system operates via a distinct neurotransmitter, providing its unique "neurochemical signature". Wakefulness-promoting neurons are active during waking and quiescent during NREM, whereas NREM-promoting neurons are active

during NREM and cease to fire during arousal. The level of arousal at any onetime reflects the intricate balance between wakefulness-promoting and NREM-promoting systems. Wakefulness is the result of cortical activation by Wakefulness-promoting neurons; sleep ensues when NREM-promoting neurons switch off the wakefulness-promoting systems.REM sleep is regulated by a network of REM-promoting and REM-inhibiting neurons located in the brainstem and hypothalamus.[38]

The wakefulness and consciousness are maintained by a continuous sensory input to the cortex from visceral as well as somatic systems through the non-specific thalamic system, sub thalamus, hypothalamus and basal forebrain. The sleep state does not result from the passive withdrawal of arousal due to fatigue of RAS. Now it is established that the sleep is produced by an active process which is different for NREM sleep and REM sleep.[38]

Genesis of NREM sleep [39,40]

The NREM sleep is generated by interaction of neurons which are grouped as:

- **Diencephalic sleep zone**: Diencephalic sleep zone lies in the hypothalamus and the nearby intra-laminar and anterior thalamic nuclei. A sleep facilitatory centre is considered to be located in the anterior hypothalamus as its stimulation causes sleep .Posterior hypothalamus acts as a waking centre as its stimulation causes wakefulness. The diencephalic

sleep zone must be stimulated at low frequency (about 8 Hz) to produce sleep.

- **Medullary synchronizing zone:** Medullary synchronizing zone is in the reticular formation of medulla oblongata at the level of nucleus of the tractus solitarius. Like diencephalic zone, this zone also produces sleep when stimulated at low frequency.
- **Basal forebrain sleep zone**: Basal forebrain sleep zone includes the pre-optic area and the diagonal band of broca. Unlike the other two zones, stimulation of this zone at low as well as high frequency produces sleep.

The NREM on cells are GABAnergic inhibitory neurons that mediate sleep inducing action of the above zones. These cells are thought to produce sleep by inhibiting the histaminergic cells in the posterior hypothalamus as well as cell of nucleus reticularispontisoralis (RPO) in the midbrain that mediate arousal.

The NREM sleep is characterized by the EEG spindles ad slow waves that are produced by synchronized post synaptic potentials in the cortical neurons. These synchronized synaptic potentials are generated by the rhythmic firing of thalamic relay neurons that project to the cortex. The rhythmic firing of relay neurons is a result of action of GABAnergic inhibitory neurons in the nucleus reticularis that forms a shell around the thalamus.

Genesis of REM sleep [39,40]

Rapid eye movement sleep is generated by the interaction of neurons in the caudal midbrain and pons with the neurons in the medulla and forebrain.

Role of cholinergic neurons of midbrain and adjacent dorsal pons

Cholinergic neurons of midbrain and adjacent dorsal pons forms an important component of the midbrain arousal system and are maximally active during waking and REM sleep. Their activity contributes to the blocking of the slow waves of EEG.[40]

Role of nucleus reticularispontisoralis (RPO)

The nucleus reticularispontisoralis forms important neuronal machinery for genesis of REM sleep.

- Cholinergic ponto-geniculo-occipital (PGO)-on cells. The discharge of these neurons produces the so called PGO spikes that are characteristics of REM sleep.
- REM -waking-on-cells of reticularispontisoralis (RPO) fire at high rate during waking as well as during REM sleep. Some of these cells project to the motor neurons that drive the extraocular muscles. so burst firing during REM sleep produces rapid eye movement and muscle twitches.
- REM-on-cells: REM-on-cells of RPO show high level of activity during REM sleep but have a very little or no activity during waking and NREM sleep.[41]

4.1.5 Sleep disorders

Roughly one third of life is spent in sleep, the time spent in sleep is essential for normal growth and development of mind and body. The disorders of sleep and wakefulness, affect daily functioning and adversely affecting health and well-being. Sleep is a biological need and nervous system cannot function for long without sleep. The effect of sleep deprivation or prolonged wakefulness shows that physical changes are few but mental changes are marked. There are no changes in the composition of blood, body temperature, only slight changes in heart rate, blood pressure and respiration. The ability to maintain equilibrium will be grossly impaired. Mental changes are marked there will be inattention, irritability, loss of memory and hallucinations and delusions. In some individuals long-term effects of sleep deprivation and sleep disorders are associated with deleterious health consequences including an increased risk of hypertension, diabetes, obesity, depression, heart attack, and stroke. Sleep loss and sleep disorders have profound and widespread effects on human health.[42,43]

4.1.5.1 Hypersomnia

Excessive sleep is known as hypersomnia**.** One form of this disorder is narcoplexy in which there is an irreversible urge to sleep during daytime. It begins often with the onset of REM sleep not preceded by slow wave sleep and lasts for about 15 minutes. The narcolepsy may also have four important co-morbid conditions: sleep apnoea, periodic

limb movements in sleep (PLMS) REM behavior disorder (RBD) and nocturnal eating disorder. The classic sleep attack is an irresistible desire to fall asleep in inappropriate circumstances and at inappropriate places. It is treated with adrenergic stimulating drugs or amphetamine like drugs.[42]

4.1.5.2 Sleep Apnea

Sleep apnea is the temporary stoppage of breathing repeatedly during sleep. Obstructive sleep apnea occurs because of obstruction in the respiratory tract. Respiratory tract obstruction is mainly due to excess tissue growth like tonsils and adenoids. Characteristic feature of sleep apnea is loud snoring. Snoring without sleep apnea is called simple snoring. But snoring with sleep apnea is serious and life threatening. Central sleep apnea occurs due to brain disorders, especially when the respiratory centers are affected. Frequent arousal from apnea events results in day time somnolence which affects the daytime concentration and performance of the individual. Sleep apnea syndrome occurs in obesity, myxedema, enlargement of tonsil and lesion in brainstem. Common features of the syndrome are loud snoring, restless movements, nocturnal insomnia, daytime sleepiness, morning headache and fatigue. In severe conditions, hypertension, right heart failure, and stroke occur.[43]

4.1.5.3 Parasomnia

Parasomnia includes several disorders that occur only during sleep. These are somnolescent starts, sensory paroxysms, nocturnal paroxysmal dystonia, sleep paralysis, night terrors and nightmares,

somnambulism (sleep walking) and REM sleep behavior disorder. Most of the parasomnias are common in children and don't require any treatment. REM sleep behavior disorder occurs more commonly in adults or older men, characterized by vigorous and often dangerous motor activity that accompanies vivid dreams. Characteristically they become aggressive, angry and shout loudly or even become violent and cause injury to themselves or to the bedmate. The violent episodes occur during the REM phase, during which hypotonia fails to occur and most of these parasomnias can be treated effectively.[44,45]

4.1.5.4 Insomnia

The chronic inability to sleep in spite of adequate opportunity to do so is called insomnia. There are two types of insomnia: **primary insomnia** in which there is abnormality in normal sleep mechanism (normal nocturnal sleep is disturbed chronically), and **secondary insomnia** in which sleep disturbances is secondary to medical (heartburn, cancer, asthma, arthritis) or psychological disorders. The insomnia may result in daytime fatigue, impaired mood and judgment, poor performance, and an increased likelihood of accidents at home and in the workplace. Sometimes insomnia is transient due to jet lag, stress, a major life change (new job or loss of a relationship), environmental factors like noise. Chronic insomnia also occurs in some individuals with insomnia minimum of three nights per week in a month or longer.[46,47]

4.1.5.5 Restless Leg Syndrome

Restless leg syndrome (RLS) is a life-long sensory motor neurological disorder that often begins at a very young age but is mostly diagnosed in the middle or later years. Patients exhibit “restlessness of their legs” as movement of the legs relieves these distressing sensations. The sensory manifestations of restless leg syndrome include intense disagreeable feelings which are described as creeping, crawling, tingling, burning, aching, cramping, knifelike or itching sensations. These sensations occur mostly between the knees and ankles causing an intense urge to move the limbs to relieve these feelings. [46-48]

4.2. Circadian rhythm

The term circadian comes from the Latin circa, meaning "around" (or "approximately"), and diēm, meaning "day". Circadian rhythms are 24 hour oscillations in many behavioral, physiological, cellular and molecular processes that are controlled by an endogenous clock which is entrained to environmental factors including light, food and stress.[49]Numerous experiments in mammals confirm that the suprachiasmatic nuclei (SCN) in the hypothalamus function as the “master clocks” that reset and synchronise the circadian rhythms of peripheral tissues. The SCN consist of neuron clusters whose electrical potential frequency fluctuates spontaneously with an approximate 24-hour periodicity.[50]

4.2.1 History

- 1729 - Jean-Jacques (French Scientist) distinguished an endogenous clock by observing the 24-hour pattern of movement of the leaves of the plant *Mimosa pudica* when kept in constant darkness.
- 1896 - Patrick and Gilbert found out the relationship between sleep deprivation and increased sleepiness.
- 1918 - Szymanski (Polish psychologist) showed the capability of animals to exhibit a 24-hour activity patterns even when external cues such as light and changes in temperature are absent.
- 1929 - Hans Berger (German psychiatrist) invented electroencephalography (EEG).
- 1937 - Alfred Lee Loomis (American scientist) recorded NREM sleep and K-complex wave.
- 1950 - Franz Halberg (American chronobiologist) coined the term 'circadian'.
- 1953 - Kleitman and Aserinkinsky (American physiologists) described REM sleep.
- 1972 - Suprachiasmatic nucleus as site of biological clock.
- 1989 - Retchtschaffen (American psychiatrist) proved that total sleep deprivation resulted in death of all rats within three weeks.
- 2007 - American Academy of Sleep Medicine classified NREM sleep into 3 stages.[49,50]

4.2.2. Anatomical organization of the biological clock

In mammals, the biological clock resides in two clusters of nerve cells called the suprachiasmatic nuclei (SCN), which are located in a region at the base of the brain called the anterior hypothalamus. The SCN plays the central role as pacemaker of circadian rhythm which coordinates the entire 24-hour functional organization of cells. The signals originating from the suprachiasmatic nucleus either neural signals which act on nerve cells or neurohormonal signals act on other organs are the physiological mechanisms underlying this coordination.[51,52]

The internal clock is entrained to maintain suitable duration of sleep and wakefulness despite inconsistency in amount of light and darkness that varies according to seasons and time zones of earth. Photoentrainment is the process by which the internal clock detects the changes in light levels and allows the body to adapt to it. Remarkably, the retinal photoreceptors that lead to visual image formation are not needed for circadian photoreception. Instead, a specialized population of intrinsically photosensitive retinal ganglion cells which contains melanopsin pigment detect light and also project directly to the SCN.[53]

Activation of the SCN evokes responses in neurons whose axons descend to the preganglionic sympathetic neurons in the lateral horn of the spinal cord. These cells, in turn, modulate neurons in the superior cervical ganglia whose postganglionic axons project to the pineal gland that is located in the midline near the dorsal thalamus.[54] The pineal gland produces melatonin (N-

acetyl-5-methoxytryptamine) that finally governs the sleep-wake cycle by regulating the brain-stem circuits.[55]

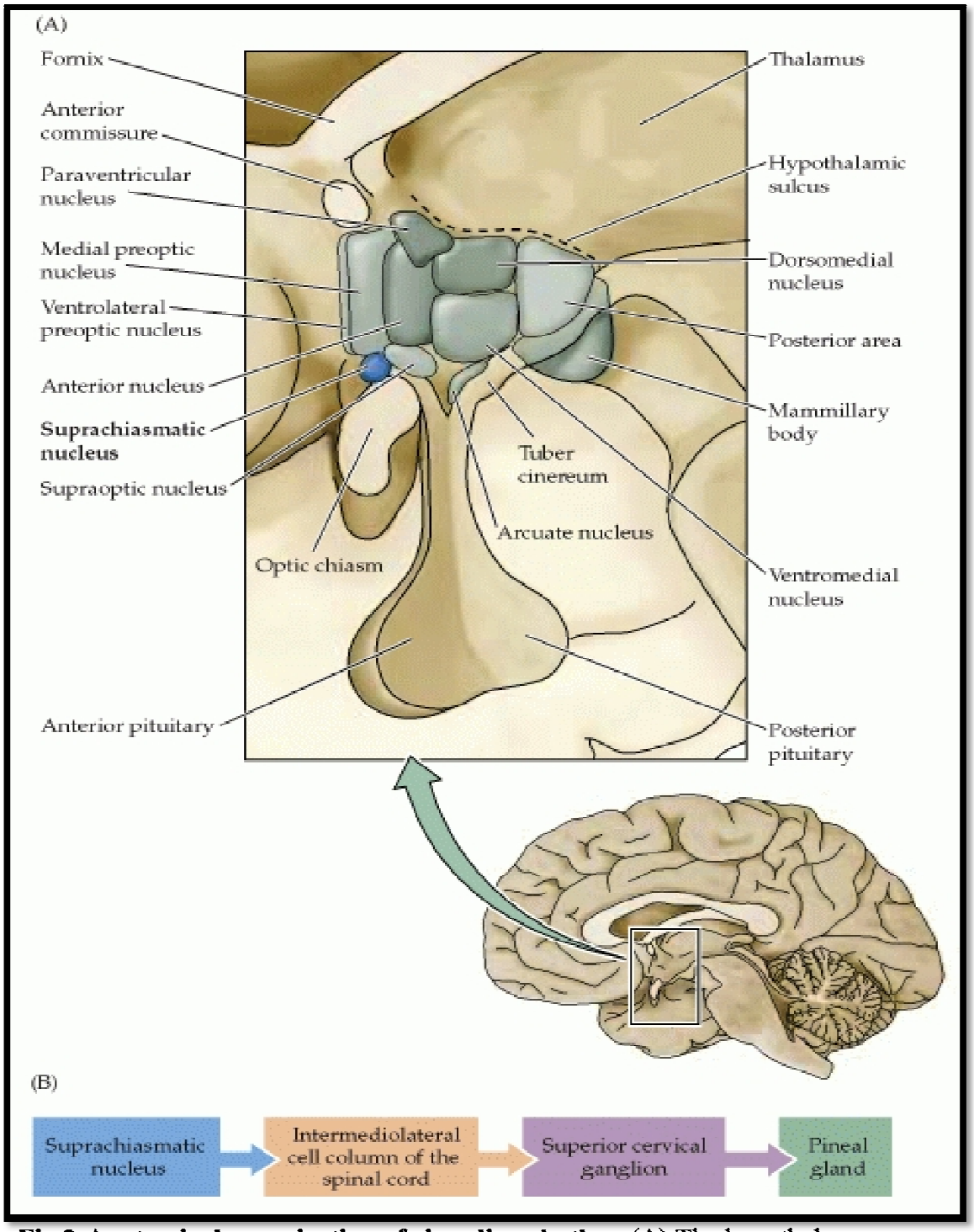


Fig 2:Anatomical organization of circadian rhythm. (A) The hypothalamus, showing the location of the suprachiasmatic nucleus (SCN). (B) Pathway from the SCN to the pineal gland.[41]

4.2.3. Neurochemical basis of circadian rhythm:

Suprachiasmatic nucleus functions with the help of neurotransmitters. The biological clock can automatically produce its own rhythms and can be entrained by the light-dark cycle. Many researches are in consistent with the finding that the presence of neurotransmitters are absolutely essential for the functioning and entrainment of SCN. Neurotransmitters like acetylcholine, glutamate, gamma amino butyric acid (GABA), neuropeptide Y (NPY), pituitary adenylate cyclase activating polypeptide (PACAP), serotonin, vasoactive intestinal peptide (VIP), and arginine vasopressin (AVP) have been implicated mainly in the functioning of the SCN. [56-58]

There are three main afferent tracts for SCN namely the retinohypothalamic tract (RHT), geniculohypothalamic tract (GHT), and afferents from nucleus raphe magnus. Three major neurotransmitters are recognized in the RHT viz glutamate, acetylcholine and PACAP.[13] Light information is imparted straight from the retina to the SCN through RHT. Photostimulation of retina induces glutamate secretion along RHT. Glutamate plays an important and critical role in mediating photic regulation of circadian rhythmicity. The second afferent tract, GHT traverses from intergeniculate leaflet (IGL) to SCN. A different branch of RHT traverses from retina to GHT. GHT functions in photoentrainment and motor activity by responding to both photic and non-photic stimulus respectively. The IGL projections to SCN contains two neurotransmitters,

NPY and GABA. The projections from nucleus raphe magnus to SCN are serotonergic neurons producing serotonin. The serotonergic projections functions in regulating photoentrainment of SCN.[57]

The pineal gland hormone, melatonin also called the "darkness hormone" is of immense importance in SCN function. This is supported by the evidence that SCN contains plenty of melatonin receptors. The purpose of melatonin is to suppress the SCN neural activity towards night time.[56-58]

The efferent projections from SCN relays information to predominantly to the hypothalamus in the preoptic, paraventricular, dorsomedial nucleus and thalamus in the paraventricular nucleus. These projections forms the basic anatomy for the control of sleep and wakeful states. AVP and VIP are the major neurotransmitters of output projections.[56-58]

4.2.4. Role of Circadian rhythm in Health and Wellness

Human performance capacity is greatly governed by the diurnal rhythms because most of the physiological and behavioral functions in man occur on a rhythmic pattern. A disturbance to this rhythmicity can be :- (1) voluntary like shift work, jet lag and (2) involuntary can have a negative impact on health and wellness. All untoward effects of disrupted rhythmicity can be related to disturbances in sleep-wake cycle.[59]

Under natural conditions, most of the animals have their timing of sleep and wakeful states synchronized to their circadian control of sleep-wakefulness. However, humans have the distinctive capability of overriding their internal biological clock control and the circadian rhythm. But this overriding may produce asynchrony between the sleep-wake cycle and circadian clock which ultimately leads to ill effects in humans.[60]

Several studies proves that abnormalities in the circadian rhythm is associated with adverse effects in humans especially affective disorders. Other study findings suggest that the two main causes of death namely myocardial infarction and stroke shows diurnal variation in occurrence. A separate branch of science - Chronotherapeutics have been developed based on the observations of various disorders to diurnal variations.[57-59,61]

4.2.5 Circadian Rhythm Sleep Disorders (CRSDs)

Sleep disorders due to disrupted circadian rhythm occurs as a result of misalignment between the biological clock and the light-dark cycle. Most of the patients presenting with sleep disorders have concomitant neurologic or psychiatric illnesses, challenging the physician in diagnosis and management. A patient is considered to have CRSD when the following three diagnostic criteria are met: (1) Sustained or repeated interruptions in sleep pattern primarily affecting the timing and duration of sleep. (2) Disrupted circadian rhythm promoting either insomnia, excessive sleepiness or both. (3) Associated impairment in social behavior and work efficiency.[62]

4.2.5.1 Primary CRSD

4.2.5.1.A Delayed Sleep Phase (DSP) Disorder

A person experience DSP when he/she constantly indulge in sleep or rise up after a delay of more than two hours than normal. These people are often called as 'evening kind' and classically stay awake until midnight or later and rise up late in the morning or in afternoon. The most important finding in DSP is that the total duration of sleep for the person is within normal range, but the individual finds hard to commence sleep before midnight and prefer to rise up late in morning or afternoon.[63]

The syndrome of DSP is proposed to have a causal relationship with various factors like biological, physiological, and hereditary. Although the total duration of sleep is normal in DSP, it can adversely affect the entrainment of the internal clock since it interferes with a person's duration of exposure to light. Moreover, dim light exposure in the late evening and at night also exerts its influence on the circadian rhythm. In persons with DSP there is a paradoxical decrease in melatonin levels due to hypersensitivity for night time exposure of bright light.[63]

Epidemiological studies reported that DSP is usually observed among teenagers with a prevalence of roughly 15%. A diagnosis of DSP is encountered in 20% of the patients presenting to sleep clinics with chronic insomnia. Additionally, almost 45% of individuals with DSP has a strong positive family history.[63,64]

DSP can be effectively managed non-pharmacologically. Treatment includes behavioral therapy for the patients helping them to resynchronize their internal clock with the 24-hr light-dark cycle. All the patients are advised to carry out a structured schedule for their sleep-wakeful cycle. Additional interventions include bright light therapy, administration of melatonin tablets, or a combination of both for resetting the internal clock.[62-65]

4.2.5.1.B Advanced Sleep Phase (ASP) Disorder

In contrary to DSP, ASP syndrome occurs when the individual constantly indulges to sleep or rise up more than 3 hours earlier than normal. These people are often referred to as 'morning kind' who classically wake up between 2-5 hours after midnight and goes to sleep between 3-6 hours before midnight. Similar to DSP, the total duration of sleep is not affected unless the individual is pre-occupied with some evening activities leading to delay in the usual early bedtime. Therefore, this syndrome has high impact on one's social and occupational commitments. The factors influencing ASP are unknown but can be attributed to biological, physiological, psychological and environmental variables. ASP is directly dependant on age and has an overall prevalence rate of 1% in elderly. [62-65]

Behavioral therapy is the cornerstone in the management of ASP syndrome while other treatment modalities like phototherapy using

bright light in the evening is also successfully used to stabilize the sleep pattern. [65]

4.2.5.1.C Irregular Sleep Wake Disorder

Indeterminate sleep-wake cycle results in irregular sleep wake disorder. The individual's sleep will be fragmented into a sequence of naps throughout the day. Clinical features include loss of concentration, lack of interest, dementia, excessive sleepiness during day, insomnia at night. Children may show mental retardation. Hence, individuals with this disorder fails to have a sound sleep instead have their sleep broken into small fragments keeping them chronic insomniacs during nighttime.[64]

Irregular sleep–wake disorder can be managed by educating the patients about sleep hygiene and helping them to achieve a normal sleep-wake cycle. Apart from proper counseling, drugs like melatonin, ramelteon-a melatonin agonist, pyridoxine can be tried. [62-65]

4.2.5.1.D. Non-24-hour Circadian Rhythm Disorder

This disorder is also known as Free running type or hypernychthemeral syndrome. The characteristic feature of this disorder is that the individual's internal clock is 'free running' or non-entrained. This may arise in certain situations where there is failure of light reaching SCN as in the case of an individual who is totally blind either congenital or acquired. This can also occur in sighted people due to chronic sleep deprivation or some other reasons. Eventually persons suffering from this disorder have their internal clock set at a

longer period than 24 hours. The clinical features include difficulty to indulge into sleep during night and excess sleepiness is observed during daytime. [62-65]

Non-blind patients can be managed with phototherapy and pharmacotherapy using melatonin. Totally blind patients can be managed with sleep aid medications. [62-65]

4.2.5.2 Secondary CRSD

4.2.5.2.A Jet Lag Syndrome

A person traveling from one time-zone to another often experiences fatigue, poor concentration and alertness, anorexia, depression. These symptoms are recognized to be as a result of disrupted biological rhythm and are called as jet lag syndrome. Severity of symptoms rely on the total number of time-zones travelled as well as the travel-direction. Whenever a person is travelling towards east, he/she finds it difficult to initiate sleep in contrast to travel towards west, he/she finds it difficult to maintain sleep. There is advancement or delay of circadian phase during east or west flight respectively. Different time-zone travelers are coerced to resynchronize their biological rhythms and this process is typically delayed by 1.5 and 1 hour a day after westbound and eastbound travel respectively. Symptoms of jet lag occurs irrespective of age and gender. However, older persons are affected more since they take more time to recuperate with the resynchronization of internal clock. Some external factors can also influence jet lag symptoms like anxiety, loss of sleep, long uncomfortable sitting positions and excessive use of coffee and alcoholic beverages.[64]

Management of jet lag symptoms comprises of reassurance of the patient and supportive measures. Prophylactic use of melatonin tablets can attenuate the condition.[66]

4.2.5.2.B Shift Work Sleep Disorder

This type of sleep disorder is often encountered whenever an individual works during his/her normal sleep period. Usually, people who do rotating shift works like doctors, nurses, security guards etc are prone for this disorder. The clinical features of this disorder are excessive sleepiness during daytime, fatigue, lethargy, irritable mood, poor ability to concentrate and diminished mental and physical performance capacity. Apart from these constitutional symptoms, gastrointestinal disturbances like altered bowel habits are very common. Many studies have also proved that the risk of cardiovascular and cerebrovascular accidents are more in shift workers. A few studies have proposed a linear association between cancer and shift work. There is a temporary increase in serum levels of serum potassium, uric acid, glucose and lipids in night shift workers. These biochemical abnormalities suggest that shift work during night hours is a catabolic state. So long-term shift work can precipitate cardiovascular and/or cerebrovascular events.

Shift work schedules can be classified into three namely:-

(a) Night Shift

(b) Evening Shift

(c) Rotating Shift

(a) Night Shift

A classical night shift is scheduled for 12 hours commencing at 8PM and terminating at 8AM. This results in sleep during day hours when most people are awake. But the quality of daytime sleep is less than nighttime sleep because of the noises and other disturbances that arises in the environment. Not only quality but also duration of daytime sleep is compromised in these individuals as they fail to maintain a sound sleep. Studies have proved beyond doubt that permanent nighttime shift workers sleep two to four hours less compared to day shift workers. This couple of hours of sleep debt can accumulate and adversely affect the individual over a succession of night shifts. The loss of sleep experienced by the person is the most in the case of night shift than evening or rotating shifts.[67]

(b) Evening Shift

This is also a 12-hour shift commencing at 2PM and ending at 8PM. Frequently called as "second shift" among workers. These second shift workers will reach home late after work and their bedtime likely to be delayed. But the total duration of sleep of second shift workers is more compared to regular day workers because they tends to get up late and do not take naps. [67]

(c) Rotating Shift

This particular group of workers do rotating day or night or evening shifts. Due to the inconsistency in their work schedule, these

people suffers the greatest sleep loss compared to other groups of shift workers. The work efficiency will also be affected since these people fails to get sufficient rest. The key factor that determines the capacity of the rotating shift worker is the frequency of rotation. Slow frequency rotation shift workers have their shift changed once in every three to four weeks. This supply ample time to adapt with their work schedule and is associated with a lesser degree of sleep debt. But, in the case of rapid frequency rotation workers where their shift changes every other day, adaption to work schedule becomes very hard with greater degree of sleep debt. [67]

4.2.5.2.C Secondary to diseases

Debilitating illness can often lead to disruption of circadian rhythm and can manifest as delayed, advanced or irregular sleep phase disorder. The clinical manifestations can be either excessive sleepiness or insomnia associated with other constitutional symptoms. The sleep quality of the patients are severely affected due to underlying medical conditions. Some conditions that can lead to disruption of biological rhythm are bronchial asthma, chronic obstructive pulmonary disease (COPD), Parkinson's disease and End-stage renal disease.[68]

4.2.5.2.D Secondary to drugs

Normal circadian rhythm can be disrupted by the use of both prescription drugs as well as illicit drug use. CNS stimulants like modafinil,

atomoxetine are being used to improve alertness and keep awake. Alcohol, opioids, barbiturates and benzodiazepines causes CNS depression.[69-71]

4.3 Evoked potentials

Evoked potential refers to the surface electrical activity recorded from the surface of the scalp in response to a specific and adequate stimulus like auditory, visual or somatosensory. Stimulation by a specific adequate stimulus produces two types electrical activity in the cerebral cortex known as primary evoked potential and diffuse secondary response.[72]

4.3.1 History

- **Emil Du Bois-Reymond (1858)** - detected the changes in potentials as the impulse passed down the nerve trunk (nerve action potential).
- **Richard Carton (1875)** – conceived the idea that as the nerve impulse flows in and out of brain its passage might be detectable. He observed cerebral potential changes evoked by sensory stimulation especially with visual stimuli.
- **Larionov (1889)** – done mapping the topographic centers of temporal cortex of a cat in response to tuning fork stimuli of different pitches.
- **Adolf Beck (1890)** – discovered the desynchronization of ongoing oscillatory activity on sensory stimulation, which is now recognized as alpha blocking of EEG. He found the positioning of electrodes which gave a response to light and a faint response to sound.

- **Pravadich Neminsky (1893)** – gave the first photograph of evoked potentials recorded from cortex of a dog following stimulation of sciatic nerve.
- **Hans Berger (1940)** - named the spontaneous ongoing activity of the brain as "DasElektrenkephalogram' and launched EEG as a clinical neurologic test
- **Dawson (1947) -** employed the neuro-electric responses to sensory stimuli that can be readily and non-invasively recorded using averaging techniques.[73]

4.3.2 Clinical significance

Stimulus related evoked potentials reflect the functional integrity of the sensory pathways from the receptor to the cortex. Therefore any delay in conduction as depicted by delayed peak or interpeak latencies would be of diagnostic value. Event related evoked potentials are related to cognitive behavior. Therefore its use in the clinical assessment of dementia and delirium is fairly well established.[74]

4.3.3 Visual Evoked Potentials

Visual evoked potentials are electrical potential differences recorded from the vertex in response to visual stimuli. The VEP's represent the mass response of the cortical and possibly sub cortical areas. Normal VEP's indicate the intactness of the entire visual system. A normal cortical response is

recorded when the entire visual pathway is normal. Responses become abnormal if there is any defect in any part of the visual system. Therefore, VEP's can only detect the abnormality ,but cannot exactly localize the site of the lesion in the visual pathway. The Visual Evoked Potentials or the Visual Evoked Responses are the evoked potentials produced when the retina is stimulated with light (flashes/pattern stimulation) and the potentials are generated in the cortical and sub-cortical visual areas and best recorded over the occipital region. It is a very important non-invasive tool in detecting visual system abnormalities.[75]

4.3.3.1 Anatomical basis

On exposure to light, the photoreceptors, rods and cones get stimulated. These receptors synapse with bipolar cells then with the ganglion cells. The axons of ganglion cells forms the optic nerve. The optic nerve is about 5 cm long and it extends from the retina to the optic chiasma. About 1 million optic nerve fibres are unmyelinated in the retina and in the optic nerve head, but get myelination as these pass through the optic chiasma located above the sella turcica.[76-78]

The optic nerve fibres primarily carry visual impulses and also impulses for accommodation and reflex response to light and other stimuli. The fibres from temporal half of the retina are located in the temporal half of the nerve and it pass through the chiasm without crossing to the opposite side and continue to the ipsilateral reflex centres for pupillary reaction and ipsilateral visual areas. The optic nerve fibres from the nasal part of retina passes through

the medial portion of optic nerve, cross at the chiasm and terminate at the contralateral cortex. In the optic chiasma, the fibres from upper retinal quadrant occupy dorsal and those from the lower retinal quadrant occupy ventral areas. The fibres associated with central vision coming from macula form papillomacular bundle in the peripheral portion of optic nerve. As the nerve approaches the chiasma, the papillomacular bundle approaches the centre of the nerve and the temporal fibres are lateral. Only the fibres from the medial half of the macula will decussate. From optic chiasma optic tract starts and end in lateral geniculate body. The optic tract consists of ipsilateral temporal and contralateral nasal retinal fibres. From the optic tract fibres responsible for pupillary reflex pass to Edinger-Westphal nucleus. The fibres carrying impulses from upper portion of retina terminate in ventromedial segment and the lower portion in ventrolateral segment of lateral geniculate body. The macular fibres occupy an intermediate position in the dorsal, middle and caudal portion of lateral geniculate body. In the lateral geniculate body, the ipsilateral temporal and contralateral nasal retinal fibres alternatively terminate in six layers. From here the neurons form optic radiation terminate in striate cortex (area 17).The macular fibres end in the occipital lobe at the pole in a wedge-shaped area. The upper half of retinal fibres relay superior and the lower half inferior to the calcarine fissure.[76-78]

4.3.3.2 Physiological basis

Activation of the primary visual cortex and surrounding areas by thalamo-cortical fibers generates the P_{100} waveform of VEP. The retinal ganglion cells are of three types: X,Y and W. The X cells are small ganglion cells that mediate the function of cone system (colour vision).They have small diameter axons and small receptive fields. They are concentrated in the central portion of visual field (central retina) and exhibit lateral inhibition. They play an important role in providing the substrate for pattern VEP through geniculate pathway. The Y cells are large ganglion cells that mediate functions of the rod system. Their axons have a large diameter with a large receptive field. They are concentrated in the peripheral visual field (peripheral retinal location) and play a salient role in the provision of substrate to flash VEP through the extra-geniculate pathway. The VEP primarily represent the activity arising from the central visual field. This can be recorded using electrodes attached to the surface of the occipital cortex. The peripheral retinal activities originating are directed towards deeper areas of the visual cortex. [76-78]

4.3.3.3 Factors affecting VEP [79,80]

(a) Physiological factors

(i). **Age** : In infants and young children, latency is longer and reaches the adult value by 5-6 yrs. In infants, amplitude is almost double of the adult value.

(ii). **Sex** : Latency is larger in males due to larger head size and low internal body temperature in males. However ,amplitude is greater in females probably due to hormonal differences.

(iii). **Hemisphere dominance**: The amplitude of VEP is greater in right hemi-field stimulation in right handed individuals due to the neuro-anatomic asymmetries of human striate cortex.

(iv). **Eye movements:** VEP amplitude is reduced by eye movements.

(v). **Visual acuity**: The latency and amplitude remain normal with visual acuity as low as 20/120; however the amplitude decreases with a further decrease of visual acuity.

(vi). **Mental activity:** Mental activity decreases latency but increases the amplitude of VEP waveform.[79,80]

(b) Technical factors

a. Screen luminance: P_{100} latency increase with decrease in brightness. The area of illumination depends upon pupillary diameter. Decrease in area of retinal illumination reduces the amplitude and increase the latency of VEP.

b. Contrast: Checkerboard pattern P_{100} wave latency and amplitude is increased and decreased respectively, when the contrast between black and white squares are reduced.

c. Size of the stimulating pattern: The amplitude of response is attenuated when the size of stimulating pattern is reduced.

d. **Check size:** Smaller the check size higher is the amplitude of response.

e. **Electrode placement:** More anterior placement of electrodes results in shorter VEP values.

f. **Frequency of stimulus:** When the stimulus frequency is more than 8/sec, the evoked responses convert into steady state evoked potentials.[79,80]

4.3.3.3 VEP Waveforms

P100 waveform generated in the striate and pre-striate occipital cortex, due to activation of primary visual cortex and thalamocortical fibers discharge. The VEP is primarily a reflection of activity originating in the central 3º to 6º of the visual field as the retinal projections from this area is relayed to the surface of the occipital lobe while those from the peripheral areas are directed to deeper regions within the calcarine fissure. Therefore, when scalp electrode picks up the signals directly from the cortical tissue that receives the central inputs. VEP is mainly a reflection of the cone activity. On giving visual stimuli, there is increased metabolism in the primary visual area and also in the visual association areas 18 and 19.The cerebral blood flow is found to increase with increase in stimulation rate up to 8 Hz but declines gradually thereafter. Using intracerebral recording in awake humans, it is found that P100 appears to be generated by the pyramidal cells in layer IV of area 17. According to the imaging studies the source of the early phase of the P100 peak appears to be in dorsal extrastriate cortex of the middle occipital gyrus, whereas the late phase of

P100 generated by the ventral extrastriate cortex of the fusiform gyrus. These results suggest the cortical generation of VEP waveforms. N70 waveform – reflects the activity in the fovea and primary visual cortex. N135 waveform – reflects the activity in the visual association area 18 and 19.[81]

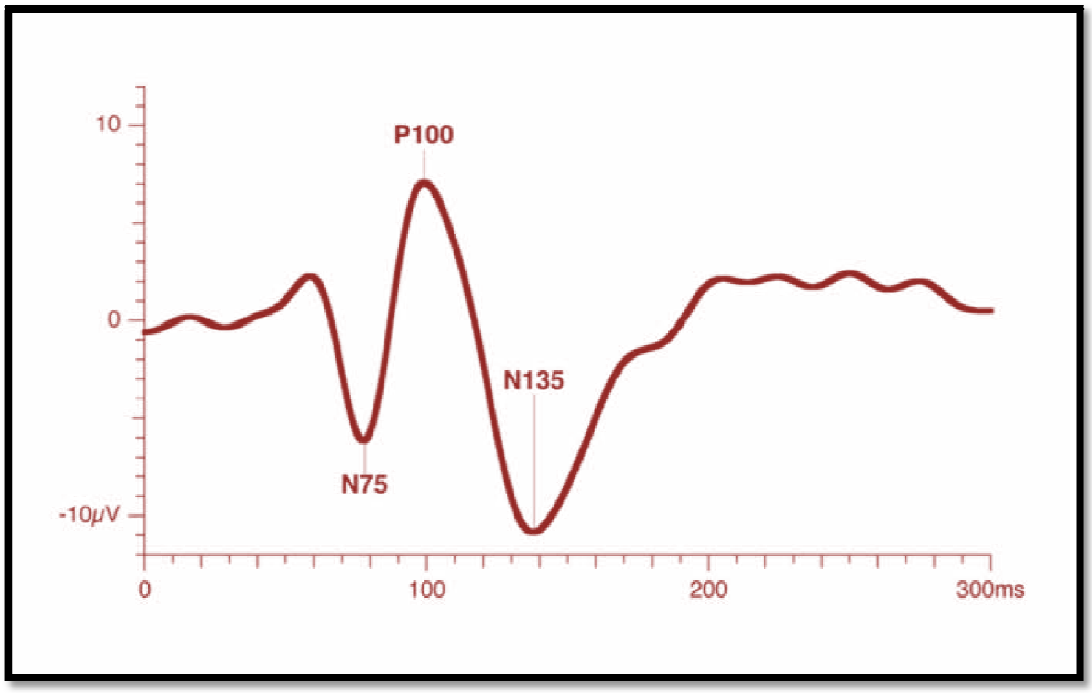


Figure 3: Visual Evoked Potential Waveform [81]

Parameter	Normal value
P100 latency (millisecs)	96.9 ±3.6
Amplitude (μV)	7.8 ±1.9

Table 1: Normal values of VEP[80]

4.3.3.4 Clinical Applications

The most important utility of VEP is in the recognition of subclinical lesions of the visual system. Asymptomatic optic neuritis is easily detected and its presence may aid in the diagnosis of multiple sclerosis. VEP is an important adjunct for the diagnosis of demyelinating diseases, since, optic

nerve abnormalities are not clearly visualized by MRI. A normal VEP doubtlessly excludes optic nerve or anterior chiasmatic lesion thus it helps to assess patients with subjective complaint of visual loss. It will also help to distinguish blindness from hysteria and malingering: if a patient reports visual loss, a normal VEP strongly favors a psychogenic disorder. In infants, VEPs has been used to assess integrity of the visual system when blindness is suspected and also to detect unilateral amblyopia at an early age when recovery may still be possible.[78]

VEP is useful in the diagnosis of Multiple sclerosis, Optic Neuritis, Ischemic Optic Neuropathy, AIDS-related ocular lesions, Toxic amblyopia, Glaucoma, Retro bulbar neuritis, Tumors compressing the optic nerve, Vitamin B12 and E deficiency, Malingering and hysteria, Intra-operative monitoring in pituitary and cavernous sinus tumor.[78,80]

Abnormalities in the VEP waveforms can be often attributed to certain clinical situations. For example :-

- ***Prolongation of latency***: Commonest cause is demyelination in optic pathways. This results in delayed conduction in the visual pathway. It may be due to multiple sclerosis, optic neuritis (alcoholism, vitamin B12 deficiency, tobacco), glaucoma.
- ***Reduction in amplitude***: Axonal loss due to ischaemic optic neuropathy produces reduction in amplitude of P100 waveform. Major causes are Hypertension, CNS vasculitis, refractory errors, media opacities, and retinal disorders.

- ***Abnormalities in both latency and amplitude***: Optic nerve compression produces segmental demyelination and axonal loss resulting in increase in latency and reduction in amplitude. This is often seen in severe papilloedema, pituitary tumour.
- ***Abnormal shape*** : Abnormality in the VEP waveforms occur in visual field defects.[78-80]

4.3.4 Brainstem Auditory Evoked Potential (BAEP)

Brainstem Auditory Evoked Potential (BAEP) constitute an objective hearing test. These are the potentials recorded from the ear and the scalp in response to a brief auditory stimulation. The evoked potentials which appear following transduction of the acoustic stimulus by the hair cells, create an electrical signal that is carried through the auditory pathway to the brainstem and from there to the cerebral cortex.[81]

4.3.4.1 Anatomical basis

The axons of the spiral ganglion that innervates hair cells of the ear from the cochlear nerve. The first order of neurons terminate in the cochlear nuclei in the medulla from where second order of neurons arise and end in the superior olivary nucleus. The third order of neurons originate from the superior olivary nucleus and ascend the lateral leminscus to project on to the inferior colliculus which is the centre for auditory reflexes. From the inferior colliculi, many fibers project to the medial geniculate body in the thalamus and from there to the primary auditory cortex.[82]

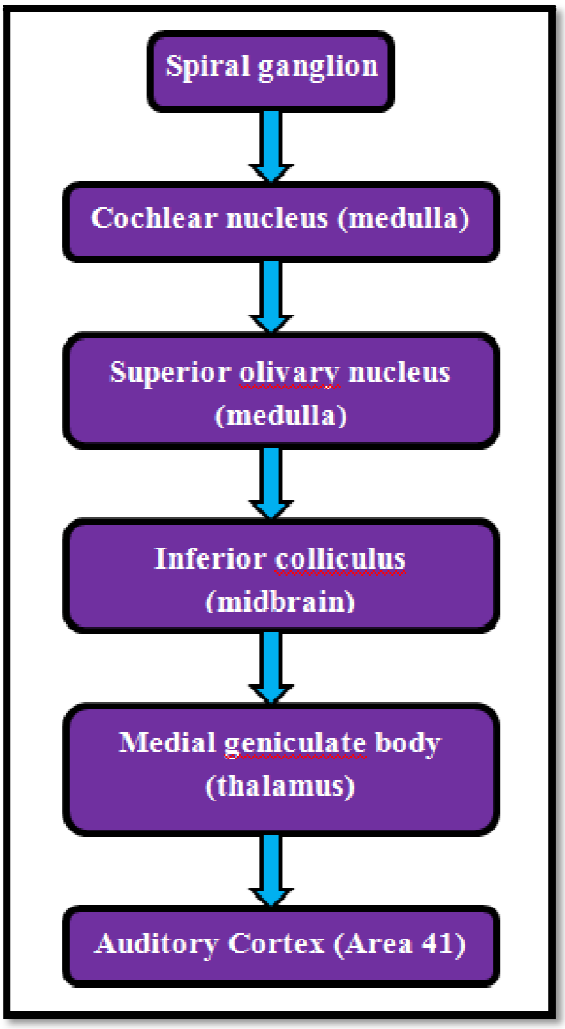


Figure 4 : Flowchart showing anatomical basis of BAEP.[82]

4.3.4.2 Physiological basis

BAEPs are recorded within 10 milliseconds after acoustic stimulus is given. The sound waves are transmitted from the external and middle ear to the inner ear. The inner ear consists of coiled cochlea containing organ of corti with the auditory receptors. The low frequency sounds affect the apical end of cochlea and high frequency sounds affect the basal end of cochlea. The amplitude of movement is directly related to the intensity of the acoustic signals. The stimulation of cochlea results in eighth nerve activity. A series of potentials

are generated corresponding to sequential activation of different parts of the auditory pathway, that is peripheral, pontomedullary, pontine and midbrain portions of the pathway. The orderly orientation of the neurons in dorsal cochlear, medial superior olivary, and lateral superior olivary nuclei results in summation of synaptic potentials to result in high amplitude electrical fields.[83-84]

4.3.4.3 Factors influencing BAEP [85-87]

(i) Technical factors

- **Stimulus intensity (click)**

When stimulus intensity decreases by about 0.03 msec/dB the absolute latencies increase and amplitudes diminishes.

- **Stimulus rate**

When the click rate increases the absolute latency of all BAEPs waves increases and amplitude of most of the waves decreases. At higher rates inter-peak latencies increases slightly.

- **Stimulus mode**

Binaural stimulation produces higher amplitude waves especially III, IV, V at all stimulus intensities than monoaural and binaural stimulation which produces a higher V/I amplitude ratio than monoaural stimulation at a given stimulus intensity.

- **Filter setting**

Filter settings affect relative amplitudes of BAEPs.

- **Reference electrode placement site**

If reference electrode is kept on the contralateral ear II-III interpeak latency decreases and IV-V interpeak latency increases.

(ii) Subject factors

- **Age**

The absolute peak latencies of the BAEP waves mainly waves I, III, and V increase with an increase in age. In subjects aged 50 years and older the absolute latencies of Waves I, III, and V are 0.1 to 0.2 ms longer than young adults. The interpeak latencies (IPLs) of the waves I-III, III-V and I-V in the older age groups had an increased value as compared to that in younger individuals.

- **Sex**

Females have shorter latency and higher amplitude compared to males. I-V IPL is shorter by 0.1 ms in females compared to males, which may be due to higher internal body temperature, differences in the hormones, and shorter length of brainstem auditory pathway.

- **Body temperature**

The absolute latency and IPL are prolonged on lowering body temperature. The wave V latency will be slightly prolonged in individuals addicted to alcohol or barbiturates because these agents produce hypothermia.

- **Hearing status**

Hearing impairment can alter the BAEPs, therefore, otoscopic examination, audiometry and hearing tests should be evaluated before conducting BAEP study.

4.3.4.4 BAEP Waveforms

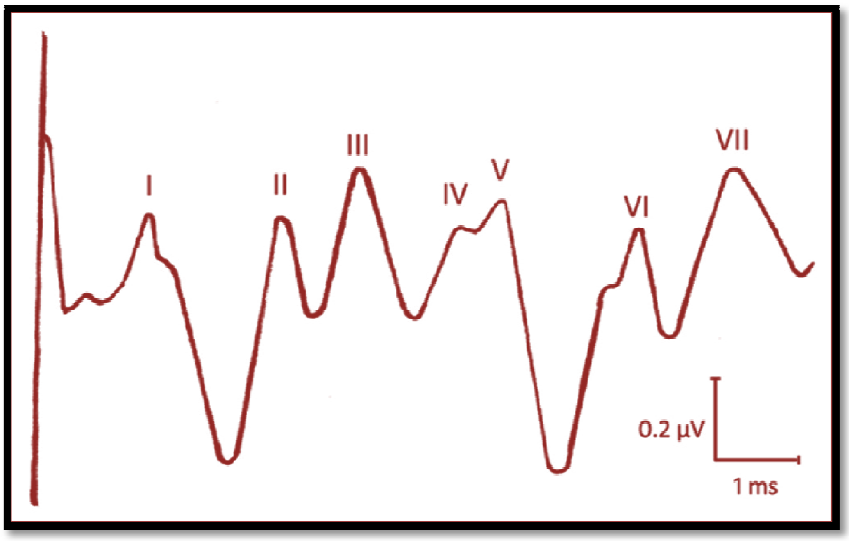


Figure 5 : Normal BAEP waveforms[84]

WAVES	ORIGIN
Wave I	Acoustic Nerve (Eighth nerve)
Wave II	Cochlear nucleus (Medulla)
Wave III	Superior olivary nucleus(Pons)
Wave IV	Lateral lemniscus (Pons)
Wave V	Inferior colliculus (Midbrain)
Wave VI	Medial Geniculate (Thalamus)
Wave VII	Auditory Radiation (Thalamocortical)

Table 3 : Site of origin of BAEP waveforms[83]

Latency	Normal value
Wave I	1.67± 0.17
Wave II	2.78 ±0.21
Wave III	3.65±0.22
Wave IV	5.0±0.30
Wave V	5.72±0.3
I- III IPL	1.99±0.25
III-V IPL	2.08±0.60
I- V IPL	4.04±0.25

Table 4 : Normal values of latency of BAEP waveforms and interpeaks[83]

4.3.4.5 Clinical applications

The most important clinical applications are to establish a minimal auditory response level, to assess the maturity of the central auditory system in neonates, to characterize the type of hearing loss, to assess the type and level of hearing loss in children below 5 years of age, to monitor surgery of the posterior fossa, to assess balance disorders, to define the site of auditory nerve or brainstem injury, to assess metabolic, demyelinating and degenerative diseases and to monitor patients in intensive care units and as a prognostic predictor of coma and determination of brain death and to detect cerebellopontine angle tumors, intrinsic brainstem tumors, multiple sclerosis, to follow -up after operations concerning brainstem etc. In the evaluation of suspected retrocochlear pathology ABR audiometry is used as an effective screening tool.[84]

The various causes for the abnormalities of BAEP includes :-

- Absence of wave I : Large tumor damaging VIII nerve, VIII nerve ischemia
- Absence of wave beyond wave I : Acoustic neuroma, meningioma, demyelinating disorders
- Absence of waves IV and V : Multiple sclerosis, hydrocephalous
- Right to left latency asymmetry more than 0.5 msec is seen in acoustic neuroma.[84,85]

4.4 Sleep Deprivation

Sleep deprivation is a very common situation encountered among professionals who fulfill crucial roles in community. It appears to cause both mental and physical ill-health.[7]

4.4.1 Etiology [4,7,10]

(i). Volitional

Seen in people who are unintentionally occupy themselves with some tasks voluntarily, resulting in limited sleep.

(ii). Self Commitments

Personal obligations can restrict the total duration of sleep and leads to sleep deprivation. e.g.:- a person may be deprived of sleep while giving care for a member of the family suffering from chronic illness.

(iii). Work

People employed in certain professions requires work hours that may lead to sleep deprivation.

(iv). Medical conditions

Most acute as well as chronic medical conditions can disturb the normal sleep physiology.

4.4.2 Impact on human health

Sleep deprivation can have a major impact on medical as well as social well being of humans jeopardizing their quality of life. The most common cause of sleep deprivation in humans is inconsistent work hours. Sleep deprivation can typically results in fatigue, as well as attenuation of alertness and cognition. There is diminished psychomotor performance and increase in both risk and incidence of errors in judgment and accidents or injuries. Sleep debt can accumulate overtime and can result in grave consequences.

Sleep deprived shift workers tends to sleep mostly during daytime. This can manifest as a negative impact to one's social behavior by missing important family get-togethers and social gatherings. All these can result in self-accusation, stress and conflicts with family and friends.[42]

4.4.3 Assessment tools [15,42]

Certain behavioral assessment tools have been developed to quantify sleep deprivation and disorders. They are as follows:-

a) Sleep Diaries or Logs

Sleep-wake diaries (sleep logs) have been used for the evaluation of timing, quantity, and quality of sleep.

b) Scales/Questionnaires

Several scales/questionnaires have been used in research works based on sleep. With the help of these questionnaires the investigator was able to quantify and measure sleepiness of an individual. Some important scales/questionnaires that are commonly used in sleep studies are as follows:-

i. Sleep Impairment Index (SII) scale

SII scale/questionnaire include five items for the assessment of severity of sleep deprivation - onset of sleep, maintenance of sleep, problems encountered after waking, interference of daytime functioning and grade of dissatisfaction with current sleep pattern.

ii. Sleep-Wake Activity Inventory (SWAI)

SWAI includes 59 questions designed to evaluate the quality of sleep as well the energy level and socializing capability of the individual.

iii. Winconsin Sleep Questionnaire (WSQ)

WSQ is specially designed questionnaire to evaluate problems during sleep like apnea, snoring, nightmares and daytime sleepiness.

iv. Pittsburg Sleep Quality Index (PSQI) Questionnaire

PSQI questionnaire is made of 19 items for the patient as well as additional 5 questions for bed companion. The questions answered by the bed companion is only taken as clinical information and is not scored. The 19 items examine the different factors that determine the quality of sleep. Seven main components of PSQI are :- (1) quality (2) latency (3) duration (4) efficiency (5) sleep alternations (6) use of sedatives (7) daytime dysfunction. Each component is scaled from 0-3 and total score ranges from 0-21. The results are interpreted as decrease in the quality of sleep as the score gets higher.

v. Leeds Sleep Evaluation Questionnaire (LSEQ)

LSEQ is a clinical research instrument used to evaluate the sleep quality before and after the treatment/study. It is visual analog scale of 10cm longitudinal line and is based on 4 items namely, (1) onset of sleep, (2) maintenance of sleep, (3) waking and (4) activities after waking.

vi. Epworth Sleepiness Scale (ESS)

It is a simple 8 item questionnaire used to evaluate sleepiness in adults. The ease with which the instrument can be applied makes it very convenient as a screening tool in clinical researches.

vii. Stanford Sleepiness Scale (SSS)

SSS is 7-point scale that is most commonly used to test alertness or degree of sleepiness. The scale has gained widespread popularity due to ease and quick way of assessing how alert the subject is.

Degree of Sleepiness	Scale Rating
Feeling active, vital, alert or wide awake	1
Functioning at high levels but not at peak; able to concentrate	2
Awake but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy, losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep; prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

c) Biomarkers

Certain biomarkers can be used to evaluate sleep loss or deprivation. They are as follows : (1) Body Temperature, (2) Melatonin (saliva, urine), (3) Cortisol (saliva, urine, plasma) (4) Plasma Growth hormone and (5) Plasma TSH. [10,15,42]

4.4.4 Effect of sleep deprivation on VEP

Hemamalini RV et al.[9] evaluated VEP in rotating night shift workers and day shift workers. A cross sectional study was conducted with eighty security guards and staff. They were divided into two groups of 40 each. Group I - Day shift workers who did not do night shift for the last 2 years and Group II - Night shift workers who had night shift for the past 6 months. The latency and amplitude of VEP between the two groups were analyzed. They found statistically significant difference in latency and insignificant difference in amplitude. Finally, they concluded that rotating night shift workers are at risk of disrupted circadian rhythm manifesting as prolonged latency of VEP.

Jackson ML et al.[15] performed a study on effects of sleep deprivation on VEP. The study was done on twenty professional truck drivers after ruling out any medical conditions. After obtaining informed consent the subjects were grouped equally into two :- Group A-deprived of sleep for 27 hours and Group B-no sleep deprivation. They found statistical significant difference in amplitude of P_{100} wave of VEP between the groups. Amplitude of P_{100} wave was reduced in Group A compared to Group B. However, there was no statistical significance in

latency of P_{100} wave of VEP between the groups although it was attenuated in Group A.

Cabrera CM et al.[88] compared the amplitude of P_{100} wave of VEP of eight adults before and after 40 hours of sleep deprivation. They revealed that the amplitude of P_{100} wave was significantly reduced when compared to the baseline value. The results of the study indicated that sleep deprivation had altered the fundamental neurophysiologic mechanisms.

4.4.5 Effect of sleep deprivation on BAEP

Jain P et al.[10] evaluated the effects of sleep deprivation on auditory event related potentials. The study was done on 34 healthy medical students of age 17-20 years. Stanford Sleepiness scale was used to assess sleep debt. The amplitude and latency of P_{300} was measured using an evoked potential measuring system. The mean baseline P_{300} amplitude was recorded as 17.33µV and after sleep deprivation was 14.27 µV . The mean baseline P_{300} latency was recorded as 311.71msec and after sleep deprivation was 297.91msec. The values when compared between before and after exposure to sleep deprivation showed statistically significant reduction in both latency and amplitude after exposure.

Harsh J et al.[89] studied auditory evoked potentials in sleep deprived subjects. The experiment was done in 40 subjects randomly divided into two groups :- Group 1 - Control (n=10) and Group 2 - Sleep deprived (n=30). The subjects in Group 2 were deprived of sleep for the last 48 hours. Auditory evoked potentials were evaluated in both the groups and compared. They observed that the latency of N2 components varied throughout the

experiment and found reduced in sleep deprived group. The reduction of latency was statistically significant compared to the control group.

Danos P et al.[90] published their preliminary results of effect of total sleep deprivation in depressed patients on the auditory event related potentials. The study was done in seventeen patients diagnosed with depression. N1, P2, N3 and P300 waves were studied. The patients were divided into two groups : Group A - Responders and Group B - Non-responders. The amplitude of N1 wave was significantly reduced in non-responders when compared to the responders group.

METHODOLOGY

4. Methodology

A descriptive study was conducted in the Department of Physiology on sixty eight (68) female shift work personnel of Sree Mookambika Institute of Medical Sciences [SMIMS], Kulasekharam, Kanyakumari District, Tamil Nadu for a period of one year from April 2015. The study protocol was approved by Institutional Human Ethics Committee [IHEC] [Ref No. SMIMS/IHEC/2015/A/30].

4.1. Inclusion and Exclusion Criteria

a. Inclusion Criteria :

1. Day shift personnel - Doctors, Postgraduates, Out-Patient(OP) staff, Nurses, Other Non-teaching staff of Sree Mookambika Institute of Medical Sciences (SMIMS) who did not do night shift in last two 2 years.

2. Night shift personnel - Duty Doctors, Interns, Postgraduates, Nurses and other Paramedical staff of Sree Mookambika Institute of Medical Sciences (SMIMS) from different wards and units namely, Intensive Care Unit (ICU), Medical, Surgical, Orthopedic emergency who did rotating night shifts for atleast 8 hours, for 6 months with one week of dayshift and one week of nightshift.

3. Age group : 20-40 years.

4. Females.

5. Normal hearing and Normal Vision.

b. Exclusion Criteria :

1. History of acute/chronic illness, neurological/psychiatric disorders.

2. History of drug intake (eg : CNS stimulants and depressants)

3. Visual defects (eg: refractive errors, colour blindness)

4. Auditory defects (eg: conductive or sensorineural hearing loss)

5. History of smoking/alcoholism.

4.2. Parameters

VISUAL EVOKED POTENTIAL (VEP) in right eye :

- Latency (Milliseconds) of P_{100}.
- Amplitude (Microvolt) of P_{100}.

BRAIN STEM AUDITORY EVOKED POTENTIAL (BAEP) in right ear :

- Peak latencies (Milliseconds) of the waves I,II,III,IV,V
- Inter-peak latencies (Milliseconds) of Waves I-III, III-V, I-V

4.3. Procedure

The study was conducted in the Research Laboratory, Department of Physiology, Sree Mookambika Institute of Medical Sciences, Kulasekharam. Written informed consent will be obtained from all the participants before enrolling them into the study. Thirty four female day shift personnel - Doctors, Postgraduates, Out-Patient(OP) staff, Nurses, Other Non-teaching staff of Sree

Mookambika Institute of Medical Sciences (SMIMS) who did not do night shift in last two 2 years and Night shift personnel - Duty Doctors, Interns, Postgraduates, Nurses and other Paramedical staff of Sree Mookambika Institute of Medical Sciences (SMIMS) from different wards and units namely, Intensive Care Unit (ICU), Medical, Surgical, Orthopedic emergency who did rotating night shifts for atleast 8 hours, for 6 months with one week of dayshift were chosen for the study after a questionnaire analysis according to the inclusion criteria. The night shift personnel were asked to report to the Physiology Research Laboratory between 8.00 AM - 9.00 AM after completing their night duty.

Baseline characteristics like age(yrs), height(cm), weight(kg), Body Mass Index(BMI), Head circumference(cm), Sitting blood pressure(mm of Hg) and Resting Heart rate (number per second) were noted. Their level of sleepiness was assessed by Stanford Sleepiness Scale.[42] All the subjects underwent an ophthalmological examination using Snellen's chart to ensure normal (6/6) vision and an auditory examination using tuning forks to ensure normal hearing. The Snellen's chart was placed 6 metres from the subjects and they were asked to read the letters in the line representing normal visual acuity (6/6).[18] Auditory examination was done by performing two tests - Weber and Rinne Test. In the Weber test, a vibrating tuning fork (256 Hz) was placed in the middle of the forehead, on top of the head equidistant from the subjects' ears on top of thin skin in contact with the bone. The subject was asked to report in which ear the sound is heard louder. A normal Weber test had a subject reporting

the sound heard equally in both sides. For the Rinne test, a vibrating tuning fork (512 Hz) was placed initially on the mastoid process behind each ear until sound is no longer heard. The fork was then immediately placed just outside the ear and the subject was asked to report when the sound caused by the vibration is no longer heard. A normal or positive Rinne test was when the sound heard outside of the ear (air conduction) is louder than the initial sound heard when the tuning fork end was placed against the skin on top of the mastoid process behind the ear (bone conduction).[73,74,76]

Visual and Auditory evoked potentials was recorded after explaining the participants about the procedure to ensure full co-operation. All recordings were carried out in day shift personnel also at the same time. Subjects were seated in an arm chair comfortably. The recordings were obtained by using the Neuropack with evoked potential measuring system (Records and Medicare systems Pvt. Ltd. Punchkula) RMS EMG Machine in an electrically and acoustically shielded room with dimmed light.[75]

Visual Evoked Potential (VEP) was tested after the electrodes were fixed with a paste in the following positions: active electrode over the occiput, reference electrode at vertex, ground electrode over the forehead. The subjects were instructed to fixate their examining eye (other being closed) on the central spot of the alternative black and white checks of the TV monitor (300 stimuli) kept at one meter distance and the signals were picked up by the electrodes. It included three waves namely; P_{100}, N_{70} and N_{135}. The P_{100} waveform of VEP was generated in the occipital cortex due to activation of primary cortex and

thalamocortical volleys. N_{70} reflected activity of fovea and primary visual cortex while N_{135} reflected the activity of visual association areas 18 and 19.[78,79,80]

Brainstem Auditory Evoked Potential (BAEP) was tested with the ground electrode placed on the forehead, active electrodes were placed on the examining mastoid and the reference electrode was placed over the other mastoid. All the electrodes were placed after cleaning the sites with spirit swabs and then affixed with an electrode paste. The signals were be picked up by these electrodes after the standard click stimuli which were delivered through the headphones, 2000 click stimuli of intensity 90dB above the normal hearing threshold was given to each ear independently. The recording consisted of I-V vertex positive (earlobe negative) waves. Wave I is believed to reflect activity in the auditory nerve; Waves II and III activity in the cochlear and superior olivary nuclei of the pons; and waves IV and V, activity in the lateral lemniscus and the inferior colliculi of the midbrain. Thus I to III inter peak latency (IPL) reflected the conduction between auditory nerve and the pons; III to V inter peak latency reflected the conduction between pontine and midbrain components of the brainstem auditory pathways. Thus inter peak latency I-V indirectly reflected neuronal conduction from acoustic nerve-pontomedullary, pontine- midbrain auditory pathways.[82,83,84] All the participant details were entered in the questionnaire and data obtained from study in Microsoft Excel 2007 and analyzed.

4.4. Statistical analysis:

The data was expressed in Mean and Standard Deviation. Statistical Package for Social Sciences (SPSS 16.0) version used for analysis. Unpaired t test applied to find statistical significance between the groups. $p<0.05$ was considered statistically significant at 95% confidence interval. The data were entered into the Microsoft Office Excel 2007 for windows 8. The results in table and bar diagrams were presented as Mean ± SD.

RESULTS

5. Results:

5.1. Study subjects:

Total number of participants recruited in this study was 34 in each group after considering inclusion and exclusion criteria. The baseline characteristics of the eligible participants were recorded and depicted in table 1.

Characteristic	Day shift personnel (n=34)	Night shift personnel (n=34)
Age (yrs)	24.68 ± 1.88	24.76 ± 1.94
Height (cm)	158.86 ± 1.52	158.56 ± 1.79
Weight (kg)	56.33 ± 0.89	56.96 ± 0.36
Body Mass Index	22.48 ± 0.04	22.51 ± 0.07
Sitting B.P (mm of Hg)	120 ± 0.67/80 ± 0.85	120 ± 0.45/80 ± 0.77
Resting HR (per min)	66 ± 1.34	67 ± 1.29
Head circumference (cm)	53.74 ± 0.98	53.88 ± 0.65

Table 1 : Baseline characteristics of the participants

[All data are expressed in Mean±SD]

5.2. Comparative assessment of mean latency (ms) of P_{100} wave in VEP between day and night shift personnel:

The study showed statistically significant ($p<0.05$) difference in the mean latency (ms) of P_{100} wave in VEP between day and night shift personnel. The changes in the latency of the two groups are given in table 2 and represented in figure 1.

Groups	Mean latency of P100(ms) wave in VEP (MEAN±SD)	p value
Day shift personnel	95.07±1.33	0.001
Night shift personnel	98.61±0.88*	

(* p<0.05 significant compared between the groups)

Table 2 : Shows comparative mean latency (ms) of P_{100} wave in VEP between day and night shift personnel

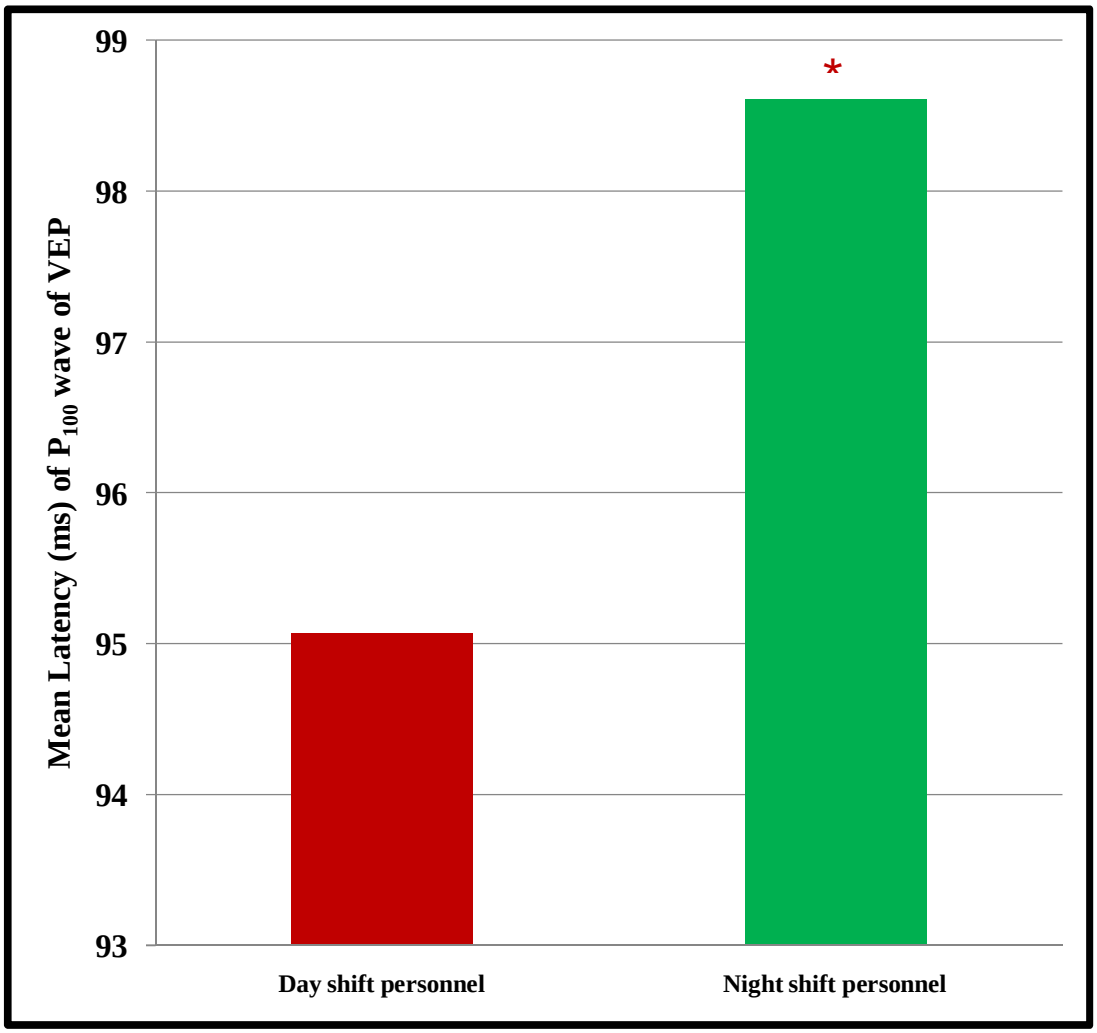


Figure 1 : Bar diagram showing comparative mean latency (ms) of P_{100} wave in VEP between day and night shift personnel.

5.3. Comparative assessment of mean amplitude (µV) of P_{100} wave in VEP between day and night shift personnel:

The study showed no statistically significant ($p>0.05$) difference in the mean amplitude (µV) of P_{100} wave in VEP between day and night shift personnel. The changes in the amplitude of the two groups are given in table 3.

Groups	Mean amplitude of P_{100}(µV) wave in VEP (MEAN±SD)	p value
Day shift personnel	7.84±0.91	0.62
Night shift personnel	7.74±0.77	

($p>0.05$ no significance when compared between day and night shift personnel)

Table 3: Shows comparative mean amplitude (µV) of P_{100} wave in VEP between day and night shift personnel.

5.4. Comparative assessment of mean latency (ms) of different waveforms of BAEP between day and night shift personnel:

The table 4 shows the comparative means of the latency (ms) of different waveforms of BAEP (Wave I,II,III,IV and V) between the two groups. Statistically significant results were obtained with the Waves III, IV and V when compared between day and night shift personnel (Figure 2). No significance was

observed when the latency of Wave I and II were compared between the two groups.

BAEP Waveforms	Mean latency (ms) (MEAN±SD)		*p value*
	Day shift personnel	Night shift personnel	
I	1.66±0.08	1.67±0.14	0.67
II	2.78±0.07	2.82±0.08	0.07
III	3.63±0.11	3.68±0.04*	**0.01**
IV	5.07±0.35	5.34±0.44*	**0.007**
V	5.44±0.27	5.85±0.36*	**0.001**

(*p<0.05 is considered significant when compared between the two groups)

Table 4 : Shows comparative mean latency (ms) of different waves of BAEP between day and night shift personnel.

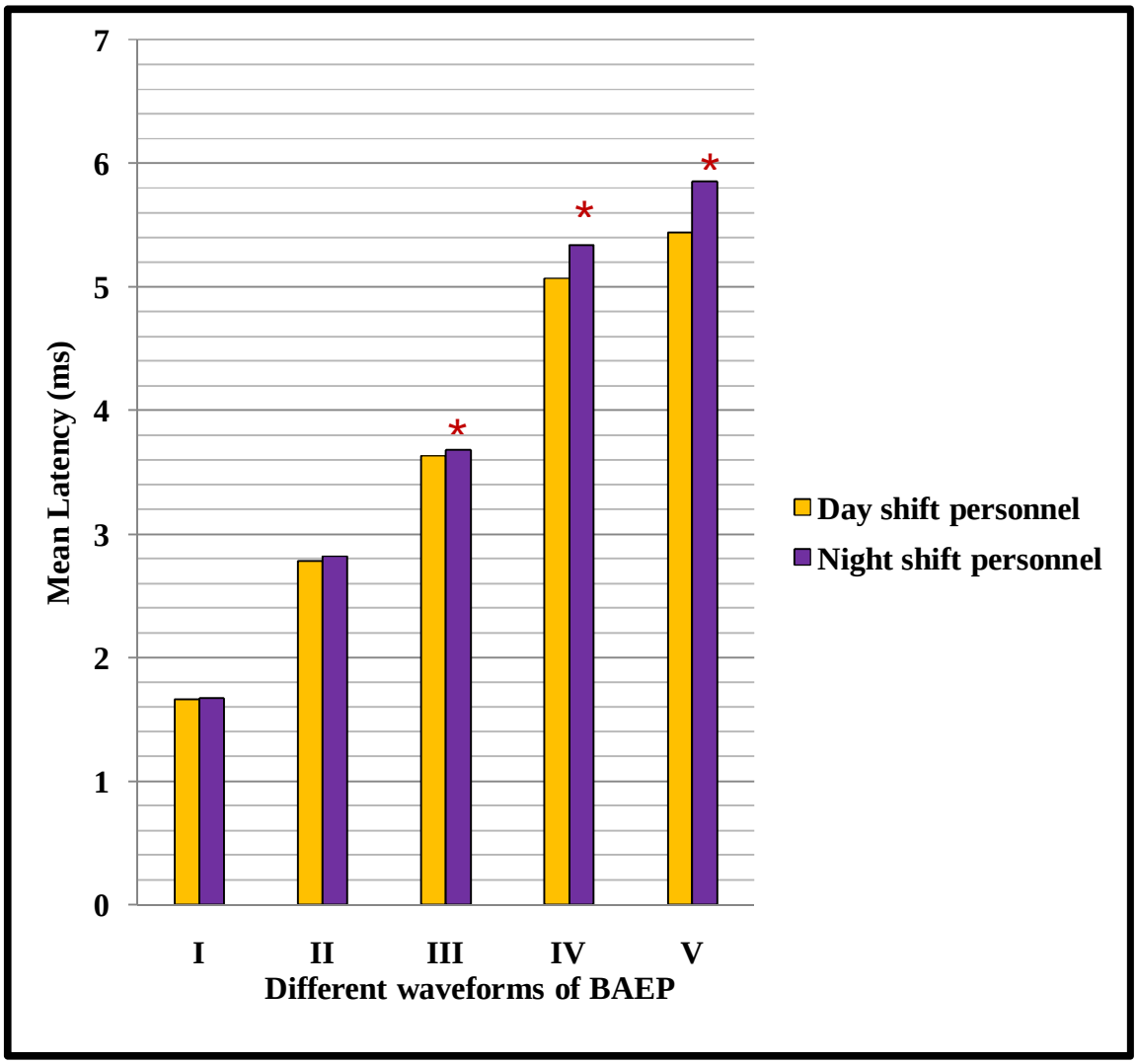


Figure 2: Bar diagram showing comparative mean latency (ms) of different waveforms of BAEP between day and night shift personnel.

5.4. Comparative assessment of mean latency (ms) of different waveforms of BAEP between day and night shift personnel:

The table 4 shows the comparative means of the interpeak latency (ms) of different waveforms of BAEP (Wave I-III, III-V and I-V) between the two groups. Statistically significant ($p>0.05$) difference were observed between the

two groups while comparing the mean interpeak latency of Wave III-V and I-V. No significance was seen between the two groups while comparing the mean interpeak latency of Wave I-III.

Type of Interpeak latencies	Mean interpeak latencies of different waves in BAEP (MEAN±SD)		*p value*
	Day shift personnel	Night shift personnel	
I-III	1.91±0.19	1.99±0.34	0.23
III-V	2.02±0.3	2.19±0.23*	**0.01**
I-V	3.96±0.29	4.11±0.33*	**0.05**

(* p<0.05 is considered significant)

Table 4 : Comparative means of interpeak latencies of different BAEP waves between day and night shift personnel.

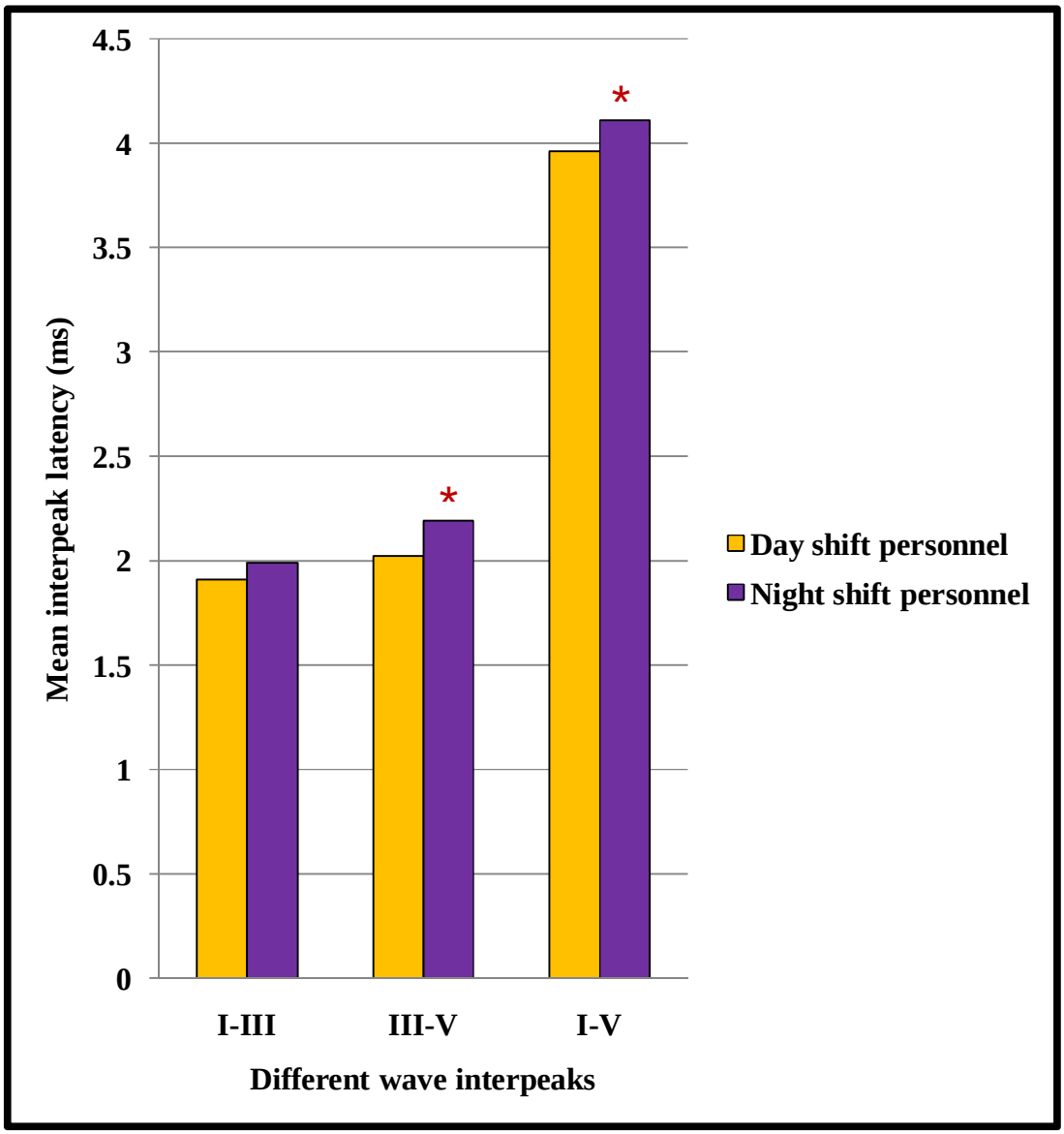


Figure 3 : Bar diagram showing comparative mean interpeak latency (ms) of different interpeak waveforms between day and night shift personnel.

DISCUSSION

6. Discussion

The present study explored the comparative changes in the Visual and Brain stem auditory evoked potentials between day and night shift personnel of a tertiary care hospital.

Evoked potentials are non-invasive method to assess the functional integrity of sensory impulse processing of central as well as peripheral nervous system. Normal VEP and BAEP indicates the intactness of the entire visual and auditory system respectively. The P_{100} waveform of VEP is generated in the occipital cortex due to activation of primary cortex and thalamocortical volleys. In BAEP, Wave I is believed to reflect activity in the auditory nerve; Waves II and III activity in the cochlear and superior olivary nuclei of the pons; and waves IV and V, activity in the lateral lemniscus and the inferior colliculi of the midbrain. Wave I to III inter peak latency (IPL) reflects conduction between auditory nerve and the pons; III to V inter peak latency reflects conduction between pontine and midbrain components of the brainstem auditory pathways. Thus inter peak latency I-V indirectly reflects neuronal conduction from acoustic nerve-pontomedullary, pontine- midbrain auditory pathways. Any subtle changes to these event related potentials can reflect the sub-clinical involvement of the central nervous system. [11,78,83.91]

Shift work sleep disorder occurs whenever a person works during his/her normal sleep time. Night shift work is commonly seen in tertiary care hospitals among doctors, nurses and other paramedical staffs for the provision of uninterrupted health care facilities to the people. A classical night shift is

scheduled for 12 hours commencing at 8PM and terminating at 8AM. This results in sleep during day hours when most people are awake. But the quality as well as duration of daytime sleep is less than nighttime sleep because of the noises and other disturbances that arises in the environment. Thus, sleep deprivation occurs in night shift workers.[45]

Sleep deprivation occurring in the night shift personnel and can be attributed to be the cause for these changes in VEP and BAEP. Sleep deprivation causes disruption of the normal sleep-wakeful cycle or circadian rhythm. The disrupted circadian rhythm can modulate the release of neurotransmitters involved in neural conduction.[92-94]

The present study was done on thirty four female day shift personnel and thirty four female night shift personnel working in a tertiary care hospital. After evaluating their degree of sleepiness using Stanford Sleepiness Scale, all participants were subjected for measurement of VEP and BAEP. The measurements obtained in both groups were compared to elicit any significant difference exists. The VEP and BAEP showed significant changes between the groups depicting the effect of sleep deprivation on the measurements.

Significant prolongation of mean latency of P_{100} wave of VEP was observed in night shift personnel when compared with day shift. Although a reduction in amplitude of P_{100} wave of VEP was observed in night shift personnel when compared to day shift, this was not statistically significant. The current study results were consistent with the study done by Hemamalini RV et al.[9] showing significant prolongation of VEP and insignificant difference in

amplitude of VEP in night shift workers compared to day shift workers. Sleep deprivation was considered to be the major cause for these changes. The present study had conflicting results with the study done by Jackson ML et al.[15] where amplitude of P_{100} wave of VEP reduced in sleep deprived individuals compared to normal individuals and latency of P_{100} wave of VEP was found to have insignificant difference between the groups.

Waves III, IV and V of BAEP showed accentuated mean latency in night shift personnel when compared with day shift. Interpeak latencies between Waves III-V and I-V also showed significant increase in night shift personnel when compared to day shift. The study results were consistent with an experiment done by Harsh et al.[89] showing increase in latency of BAEP waves in sleep deprived individuals compared to the control group.

The prolongation of latency of waves of VEP and BAEP directly correlates to slowing of neural conduction due to sleep deprivation in night shift workers compared to day shift. This slowing of neural conduction could be considered as a very early diagnostic marker of dysfunction of normal visual and auditory physiology. Many studies have also proved that the risk of cardiovascular and cerebrovascular accidents are higher in shift workers. Also few studies have proposed a linear association between cancer and shift workers too. Some biochemical abnormalities like increased levels of glucose, uric acid, potassium and lipids suggests that night shift work is a catabolic state.[88-90]

The main limitation of this study is that the assessment of sleep deprivation was subjective. Only female day and night shift personnel were included in the study.

Further studies are required for supporting this evidence of subclinical involvement of the integrity of vision and hearing physiology due to night shift work.

CONCLUSION

7. Conclusion:

Significant prolongation of mean latency of P_{100} wave of VEP in night shift personnel when compared with day shift. No significant changes observed in the amplitude of P_{100} wave of VEP between both day and night shift personnel. Waves III, IV and V of BAEP showed increased mean latency in night shift personnel when compared with day shift. Interpeak latencies between Waves III-V and I-V showed significant increase in night shift personnel when compared to day shift.

SUMMARY

8. Summary:

Health care personnel working in tertiary care hospitals have inconsistent work hours like night shift for uninterrupted care for the community. These night shift workers are more prone for sleep deprivation as a result of their fluctuations in their normal sleep time. Sleep deprivation among them leads to excessive daytime sleepiness and reduced alertness. The negative impact of sleep loss extends to the subclinical involvement of visual and auditory physiology as implicated by changes observed in Visual and Auditory Evoked potentials.

This comparative study was done in sixty eight female shift workers in a tertiary care hospital. They were divided into two groups of thirty four participants each. First and second group comprised of day shift and night shift personnel. VEP and BAEP was measured in all the participants. The results were compared between the two groups.

The measurement of amplitude and latency of P_{100} wave in VEP is a simple and non-invasive method to test the integrity of the visual pathway. Any changes in the measurements correlates to disruption of normal visual physiology. In this study, the latency of P_{100} wave in VEP was prolonged in night shift personnel working in a tertiary care hospital compared to day shift. This can be explained due to the alternation in the circadian rhythm owing to sleep deprivation in night shift personnel. The observations suggest that there is a subclinical involvement of the visual pathway functioning in night shift personnel.

The measurement of latency and inter-peak latency of waves of BAEP is an ideal method for the evaluation of integrity of the auditory pathway. Any subtle changes in the measurements shows a linear association with altered auditory physiology. In this study, the latency of waves III, IV, V and inter-peak latency of waves III-V, I-V were prolonged in night shift personnel working in a tertiary care hospital compared to day shift. This prolongation of latency is proposed to be due to delayed neural conduction in night shift workers as a result of disrupted circadian rhythm. The altered circadian rhythm can modulate the release of neurotransmitters in the brain.

The study concluded with the findings that night shift personnel working in a tertiary care hospital have decreased alertness, excessive daytime sleepiness and prolongation of the latency of VEP and BAEP waves. Further studies are required to find the effects of long term sleep deprivation in normal functioning of visual and auditory pathway.

REFERENCES

9. REFERENCES

1. Watson NF, Safwan BM, Belenky G. Recommended amount of sleep for a healthy adult: A joint consensus statement of the American Academy of Sleep Medicine and Research Soceity. J Clin Sleep Med 2015;11(6):591-2.
2. Dongen HPA, Rogers NL, Dinges DF. Sleep debt : Theoretical and empirical issues. Sleep Biol Rhythms 2003;1:5-13.
3. Boonstra TW, Stins JF, Dafferstshofer A. Effects of sleep deprivation on neural functioning - an integrative review. Cell Mol Life Sci 2007;64:934-46.
4. Lim J, Dinges DF. A Meta-analysis of the impact of short term sleep deprivation on cognitive variables. Psychol B 2010;136:375-89.
5. Spiegal K, Leprault R, Cauter EV. Impact of sleep debt on metabolic and endocrine function. Lancet 1999;354:1435-9.
6. Gumenyuk V, Howard R, Roth T. Sleep loss, circadian mismatch and abnormalities in reorienting of attention in night workers with shift work disorder. J Sleep 2014;37:545-56.
7. Sauvet F, Letheriotis Georges, Merino DG. Effect of acute sleep deprivation on vascular function in healthy subjects. J Appl Physiol 2010;108:68-75.
8. Rajaratnam S, Howard ME, Grunstein RR. Sleep loss and circadian disruption in shift work : Health burden and management. Med J Australia 2013;199(8):11-5.
9. Hemamalini RV, Krishnamurthy N, Saravanan A. Influence of Rotating Shift Work on Visual Reaction time and Visual Evoked Potential. J Clin Diagn Res 2014;8:4-7.
10. Jain P, Mahajan AS, Babbar R. Effect of partial sleep deprivation on Auditory Event Related Potential and Reaction time in medical students. J JK Sci 2010;12:19-22.
11. Tandon OP. Average Evoked Potentials - Clinical Applications of Short Latency Responses. Indian J Physiol Pharmacol 1998;42:172-88.

12. Nakagawa H, Okumura N. Coordinated regulation of circadian rhythms and homeostasis by the suprachiasmatic nucleus. Proc Japan Acad Ser 2010;86:391-409.
13. Schmidt TM, Chen SK, Hattar S. Intrinsically photosensitive retinal ganglion cells: many subtypes and diverse functions. Trends Neuro Sci 2011;839(2):1-9.
14. Namita, Ranjan DP, Shenvi DN. Effect of shift working on reaction time in hospital employees. Indian J Physiol Pharmacol 2010;54:289-93.
15. Jackson ML, Croft JR, Owens K. The Effect of Acute Sleep Deprivation on Visual Evoked potentials in Professional Drivers. J Sleep 2008;31:1261-9.
16. Chokroverty S. Overview of sleep and sleep disorders. Indian J Med Res 2010;131:126-40.
17. Chokroverty S. An overview of normal sleep. In: Chokroverty S, editor. Sleep disorders medicine: Basic Science, technical considerations and clinical aspects, 3rd ed. Philadelphia : Elseiver:Butterworth;2009.
18. Caeskadon MA, Dement WC. Monitoring and staging human sleep; Principles and practice of sleep medicine,2011. 5thedition. St.Louis; Elsevier Saunders:16-26.
19. Rechtschaffen A, Bergmann BM. Sleep deprivation in the rat : An update of the 1989 paper. Sleep 2002;25(1):18-24.
20. Vyazovshiy VV, Delogu A. NREM and REM sleep : Complementary roles in recovery after wakefulness. Neurosci 2014;10(52):1-17.
21. Gais S, Lucas B, Born J. Sleep after learning aids memory recall. Learn Mem 2006;13:259-62.
22. Susmakova K. Human sleep and sleep EEG : Measurem Sci Rev 2004;4(2):59-74.
23. Rosenthal MS. Physiology and neurochemistry of sleep. American J Pharma edu 1998;62:204-8.
24. Floras JS, Jones N, Johnston JA. Arousal and the circadian rhythm of blood pressure. Clin Sci Mol Med Suppl 1978;55(4):395-417.

25. Iber C, Ancoli-Israel S, Chesson AL. The AASM Manual for the scoring of sleep and associated events : Rules, Terminology and Technical specifications. Westchester IL : American Academy of Sleep Medicine 2007.
26. Takeuchi T, Ogilvie RD, Murphy TI, Ferrilli AV : EEG activities during elicited sleep onset REM and NREM periods reflect different mechanism of dream generation. Clinical Neurophysiology 2003;114:210-20.
27. Bader G, Gilberg C, Johnson M. Activity and sleep in chidren with ADHD. Sleep 2003;136:26-32.
28. Dement W, Kleitman N. Cyclic variations in EEG during sleep and their relation to eye movements, body motility and dreaming. Electroencephalo Clin Neuro Physiol Suppl 1957:9(4):673-90.
29. Madsen PL, Holm S, Vorstrup S. Human regional cerebral flow during rapid eye movement sleep. J Cereb blood flow Met 1991;11(3);502-7.
30. Parker KP, Dunbar SB. Cardiac nursing in sleep. 2005. Philadephia 5th ed : 197-219.
31. Keyger MH, Roth T, Dement WC, editors. Principles and Practice of Sleep Medicine. Philadelphia : Saunders;2005.
32. Luppi PH, Fort P. Neurochemistry of sleep. In: Montagna P, editor. Handbook of Clinical Neurology. Elseiver:Amsterdam;2010.
33. Belisio AS, Louzada FM, DeAzevedo CV. Influence of social factors on the sleep wake cycle in children. Sleep Sci 2010;3(2):82-6.
34. DeSousa IC, Louzada FM, DeAzevedo CV. Sleep-wake cycle irregularity and daytime sleepiness in adolescents on schooldays and on vacation days. Sleep Sci 2009;2(1):30-5.
35. Lima PF, Medeiros ALD, Araujo JF. Sleep-wake pattern of medical students : early versus late class starting time. Brazilian J Med Bio Res 2002;35:1373-7.
36. Maire M, Reichert CF, Schidmt C. Sleep-wake rhythms and cognition. J Cogn Behav Psycho Ther 2013;13:133-70.

37. Korf HW, Beck FF, Clasca F. Functional anatomy of sleep-wakefulness-cycle. Berlin : Springer 2011. Pg.63-104.
38. Rechtschaffen A. Current perspectives on the function of sleep. Perspect Bio Med 1998:41(3):359-80
39. Pal D, Mallick BN. Neural mechanism of rapid eye movement sleep generation with reference to REM-off neurons in locus coeruleus. Indian J Med Res 2007;721-39.
40. Squire L, Berg D, Bloom F. Fundamental Neuroscience. 3rd ed.Canada:Elsevier;2008. Pg.931-58.
41. Purves D, Augustine GJ, Fitzpatrick D et al.,editors. Neuroscience. 2nd ed. Sinauer Associates;2001.Pg 594-621.
42. Colten HR, Bruce M, editors. Sleep disorders and sleep deprivation: An umet public health problem. 1st ed. The Nat Academies press : Washington DC 2006. Pg 21-2.
43. Mohsen EM, Mohammed MA, Almezaien MM, Sobh DET. Factors associated with sleep pattern disturbance among patients in critical care units. J Nur Health Sci 2015;4(2):54-63.
44. Culebras A, editor. Sleep disorders and neurological disease. New York : Marcel Dekker 2005. Pg. 1-32.
45. Pollak CP, Thorpy MJ, Yager J. The encyclopedia of sleep and sleep disorders. New York : Infobase Publishing 2010. Pg. 111-20.
46. Kumar VM. Sleep and sleep disorders. Indian J Chest Dis Allied Sci 2008;50:129-35.
47. Lovell K, Liszewski C. Normal sleep pattern and sleep disorders. American Academy of Sleep Medicine. International classification of sleep disorders, Revised : Diagnostic and coding manual. Chicago 2011.
48. Chokroverty S, Montagna P. Sleep, breathing and neurological disorders. In : Chokroverty S, editor. Sleep disorders medicine : Basic science, technical

considerations and clinical aspects. 3rd ed. Philadelphia:Elseivier Butterworth;2009.

49. Sehgal A. molecular biology of circadian rhythm. In. Genetic Basis for circadian rhythms in mammals. 2004. Alvarez JD edt. USA : 17-33.
50. Borbely AA, Achermann P. Concepts and Models of Sleep regulation : An overview. J Sleep Res 1992;1:63-79.
51. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. Nature publishing group 2005. 437(10):1257-61.
52. Barrett KE, Barman SM, Boitano S, Brooks HL. Ganong's Review of Medical Physiology. 23rd ed. New Delhi: Tata McGraw-Hill; 2010. p. 270-80.
53. Toh KL. Basic science review on circadian rhythm biology and circadian sleep disorders. Ann Acad Med Singapore 2008;37:662-8.
54. Olds W, editor. Sleep, circadian rhythm and metabolism : The rhythm of life. Toronto: Apple Academic Press 2015. Pg.11-20.
55. Vitaterna MH, Takakashi JS, Turek FW. Overview of circadian rhythms. Alcohol Res Health 2001;25(2):85-93.
56. Card JP, Moore RY. The suprachiasmatic nucleus of the golden hamster : Immunohistochemical analysis of cell and fiber distribution. Neuroscience 1984;13:415-31.
57. Vandenpol AN. Gamma-aminobutyrate, gastrin releasing peptide, serotonin, somatostatin and vasopressin : Ultrastructural immunocytochemical localization in presynaptic axons in the suprachiasmatic nucleus. Neuroscience 1986;17:643-59.
58. Rusak B, Bina KG. Neurotransmitters in the mammalian circadian system. Ann Rev Neurosci 1990;13:387-401.
59. Borbely AA. From slow waves to sleep homeostasis new perspectives. Archiv Italienne de Biol 2001;139:53-61.
60. Borbely AA, Achermann P. Sleep homeostasis and models of sleep regulation. J Bio Rhyt 1999;14(6):559-68.

61. Jenni OG, Achermann P, Carskadon MA. Homeostatic sleep regulation in adolescents. Sleep 2005;28(11):1446-54.
62. Rosenwaser AM, Turek FW. Physiology of mammalian circadian rhythm. Sunderlands : Sinaeur Associates 2003. Pg.351-62.
63. Martinez D, Lenz MDCS. Circadian rhythm sleep disorders. Indian J Med Res 2010;131:141-9.
64. Venkatramanujam S, Jarnail S, Perumal P et al. Jet lag, circadian rhythm sleep disturbances and depression : the role of melatonin and its analogs. Adva Ther 2010;27(11):796-813.
65. Boulos Z, Campbell SS, Lewy AJ et al. Light treatment for sleep disorders : Consensus report on jet lag. J Biol Rhyt 1995;10(2):167-76.
66. Szabadi E. Neuronal network regulating sleep and arousal : Effect of drugs. Guglietta A (ed.). Drug treatment of sleep disorders, milestones in drug therapy: Springer 2015:25-16.
67. Knutsson A. Health disorders of shift workers in-depth review : Shift work. Occup Med 2003;53:103-8.
68. Keele CA, Neil E, Joels N. Samson Wright's Applied Physiology. 13thed. New Delhi: Oxford Medical Publications; 1982. p. 563-99.
69. Jain Ak. Textbook of Physiology. Vol 2. 4thed. New Delhi: Avichal publicating company; 2009. p.821-35.
70. Reghunandanan V, Reghunandanan R, Mariya RK. Vasopressin : Its possible role in circadian time keeping. Chronobiologia 1991;18:39-47.
71. Campbell SS, Gillin JC et al. Gender differences in circadian temperature rhythms of healthy elderly subjects..1989:Relationship to sleep quality 12(6) : 529-36.
72. Hall JE, Vaz M, Kurpad A, Raj T. Guyton and Hall Textbook of Medical Physiology 12thed. A South Asian Edition. 1sted. New Delhi. Elsevier; 2013. p. 822-5.

73. Misra UK, Kalita J. Clinical Neurophysiology. 2nd ed. New Delhi: Elsevier; 2006. p. 309-27.
74. Jain Ak. Manual of Practical Physiology for MBBS. 1st ed. New Delhi: Arya publications; 2004.p. 254-73.
75. Phurailatpam J. Evoked potentials: Visual evoked potentials (VEPs): Clinical uses, origin, and confounding parameters. J Med Society .2014;28 :140
76. Pal GK, Pal P, Nanda N. Text book of Medical Physiology. 2nd ed. New Delhi: Ahuja Publishing house; 2007. p. 461-83.
77. Tandom OP, Tripathi Y. Best and Tailor's Physiological basis of medical practice.13th ed. Haryana: Lippincott; 2012. p. 954-71.
78. Walsh P, Kane N, Butler S. The Clinical Role Of Evoked Potentials. J NeurolNeurosurg Psychiatry 2005;76(Suppl II):16–22.
79. Odom JV, Bach M, Brigell M, Holder GE, Daphne L, McCulloch etal.ISCEV standard for clinical visual evoked potentials (2009 update). Doc Ophthalmol 2010; 120:111–119.
80. American Clinical Neurophysiology Society (2008) Guideline 9B: Guidelines on Visual Evoked Potentials .Recommended Standard For Visual Evoked Potentials.Available at http://www.acns.org/pdf/ guidelines/ Guideline-9B.pdf (Last accessed on August 3rd 2016).
81. Emerson RG, Pedley TA. Clinical Neurophysiology Electroencephalography and Evoked potentials. In:Daroff RB, Fenichel GM, Jankovic J ,Mazziotta JC. Bradley's Neurology in Clinical Practice.Vol1. 6th ed. China: Elsevier; 2008.p. 368-393.
82. Zaher A.Visual and Brainstem Auditory Evoked Potentials in Neurology. In:Schwartz M. EMG Methods for Evaluating Muscle and Nerve Function. Vol 1. 1st ed. Europe: Intec; 2012. p.281-310
83. Picton TW, Woods DL, Braun JB, Healey TMG. Evoked Potential Audiometry. J Otolaryngol 1977:6:90-119.

84. Esteves MC, Aringa AHBD, Arruda GV, Nardi JC. Brainstem Evoked Response Audiometry In Normal Hearing. Braz J Otorhinolaryngol 2009;75:420-5.
85. Harinder J S, Ram Sarup S, Sharanjit K. The study of age and sex related changes in the brainstem auditory evoked potential. J ClinDiagn Res 2010;4:3495-9.
86. Khatoon M,Nighute S, Awari A, Ishaque M. The Influence Of Aging On Auditory Evoked Potential In: Advanced Age Group IJBR 2012; 3[11]:422-6.
87. Lotfi Y, Abdollahi FZ.Age and Gender Effects on Auditory Brain Stem Response (ABR).Iranian Rehabilitation Journal 2012;10:30-6.
88. Cabrera CM, Arce C. Amplitude reduction in visual event-related potentials as a function of sleep deprivation. Sleep 1999;22(2):181-9.
89. Harsh J, Badia P. Auditory evoked potentials as a function of sleep deprivation. J Work Stress 1989;3(1):79-91.
90. Danos P, Kasper S, Scholl HP. Clinical response to sleep deprivation and auditory-evoked potentials--preliminary results. Pharmacopsychiatry 1994;27(2):70-1.
91. Berne RM, Levy MN, Koeppen BM, Stanton BA. Physiology. 5th ed. United States of America: Mosby; 2004. p. 193-97.
92. Hannibal J. Neurotransmitters of the retinohypothalamic tract. Cell Tissue Res 2002;309:73-88.
93. Moorey RY. Organization and function of a central nervous system oscillator : the suprachiasmatic nucleus. Fed Proc 1983;42:2783-89.
94. Reghunandanan V, Reghunandanan R. Neurotransmitters of the suprachiasmatic nuclei. J Circad Rhy 2006;4(2):1-20.

ANNEXURES

Annexure 1 : Institutional Human Ethics Committee Certificate

Sree Mookambika Institute of Medical Sciences
Kulasekharam (K.K District, TN) 629161
Phone No: 04651-280866, Fax No. 04651-280740

Institutional Human Ethics Committee
Registered under CDSCO with Reg No. ECR/446/Inst/TN/2013

Ref. No. SMIMS/IHEC/2015/A/30 Date: 10th April 2015

Certificate

This is to certify that the Research Protocol Ref. No. **SMIMS/IHEC/2015/A/30**, entitled "Effect of Sleep Deprivation on Visual and Auditory Evoked Potentials in Female Night Shift Personnel of a Tertiary Care Hospital" submitted by Dr. Archana Chandran, Postgraduate of Department of Physiology, SMIMS has been approved by the Institutional Human Ethics Committee at its meeting held on 13th of March 2015.

[This Institutional Human Ethics Committee is organized and operates according to the requirements of ICH-GCP/GLP guidelines and requirements of the Amended Schedule-Y of Drugs and Cosmetics Act, 1940 and Rules 1945 of Government of India.]

10/4/15

Dr. Rema Menon. N
Member Secretary
Institutional Human Ethics Committee
Professor of Pharmacology and HOD
SMIMS, Kulasekharam [K.K District]
Tamil Nadu -629161

Annexure 2: Consent Form

CONSENT FORM

PART 2 OF 2

PARTICIPANTS CONSENT FORM

The details of the study have been explained to me in writing and the details have been fully explained to me. I am aware that the results of the study may not be directly beneficial to me but will help in the advancement of medical sciences. I confirm that I have understood the study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I have been given an information sheet giving details for the study. I fully consent to participate in this study titled **"Effect of Sleep Deprivation on Visual and Auditory Evoked Potentials in female night shift personnel of a tertiary care hospital.".**

Serial no/Reference No:

Name of the Participant:

Address of the Participant:

Contact no of the Participant:

Witnesses:

1.

2.

Place: Kulasekharam Signature/ Thumb impression of the participant

Date:

Annexure 3 : Case Record Form

NAME :

AGE : yrs

HEIGHT : cm WEIGHT : kg BMI :

BLOOD PRESSURE (SITTING) : / mmHg Resting Heart Rate : / min

HEAD CIRCUMFERENCE : cm

Please put a (✓) tick mark on the most relevant answer for each question given below :

1. Have you taken any night shift work during the past two years ?

   YES ☐ NO ☐

2. If yes, have you worked for at least six months with one week of dayshift and one week of nightshift ?

   YES ☐ NO ☐ NOT APPLICABLE ☐

3. Do you wear glasses or contact lens for any problems related to eye sight ?

   YES ☐ NO ☐

4. Do you need any hearing-aids for normal hearing ? YES ☐ NO ☐

5. Do you take any medications regularly? YES ☐ NO ☐

6. If yes, please mention the condition for which the medication is used and/or name of the medication ?
   ……
   ……
   ……...

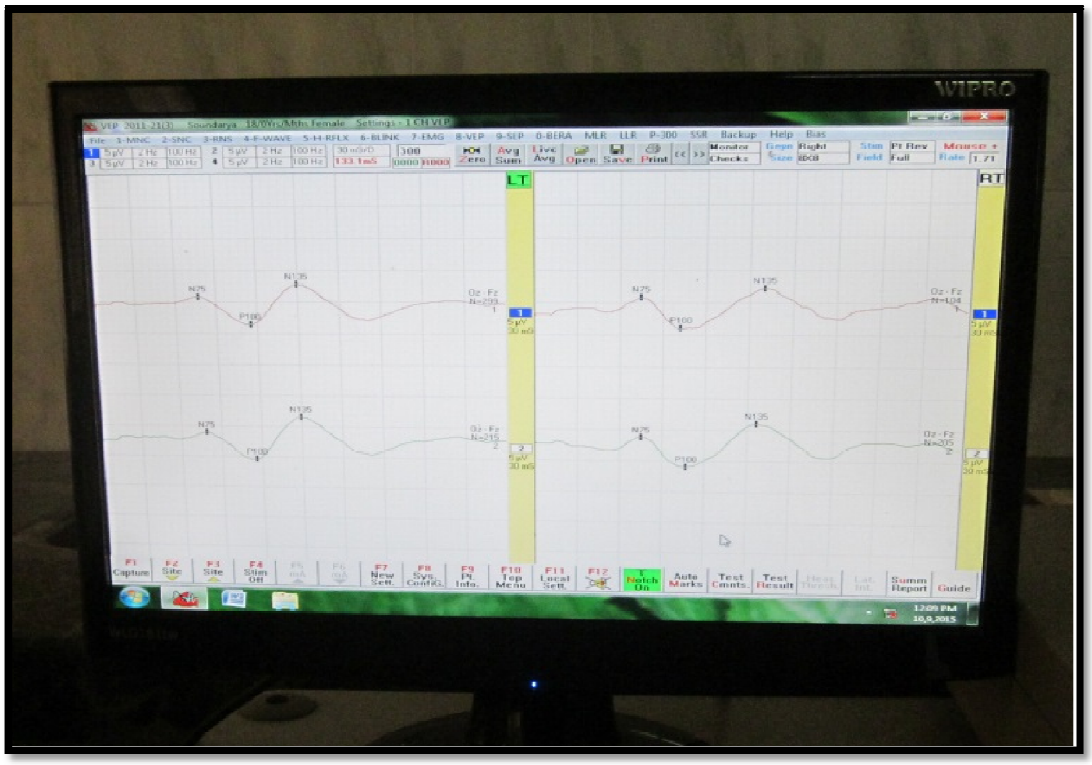
7. Do you smoke ? YES ☐ NO ☐

8. Do you consume alcohol ? YES ☐ NO ☐

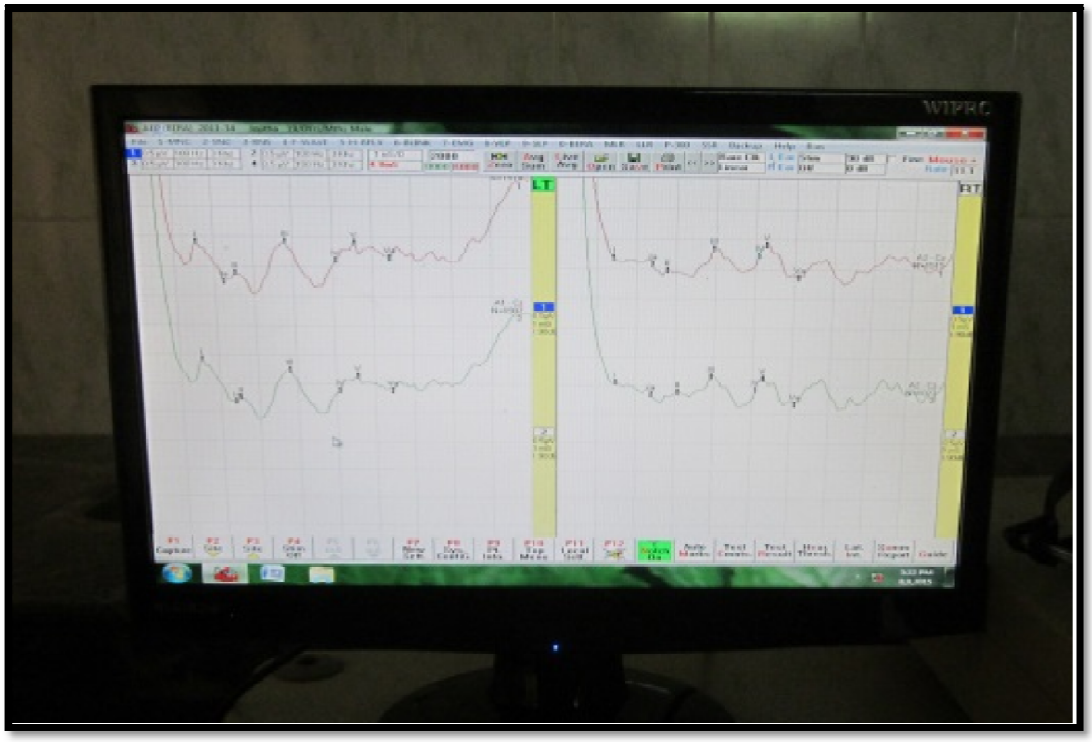
Annexure 4: Computerized Visual Evoked Potential (VEP) Measuring System.

Annexure 5: Computerized Brainstem Auditory Evoked Potential (BAEP) Measuring System.

Annexure 6: Recorded VEP waveforms



Annexure 7: Recorded BAEP waveforms



List of abbreviations

µV	microvolt
ABR	Auditory Brainstem Response
ASP	Advanced Sleep Phase
AVP	Arginine Vasopressin
BAEP	Brainstem Auditory Evoked Potential
cm	centimeter
CNS	Central Nervous System
CRSD	Circadian Rhythm Sleep Disorder
dB	decibel
DSP	Delayed Sleep Phase
EEG	Electroencephalography
EMG	Electromyography
EOG	Electrooculography
EP	Evoked Potential
GABA	Gamma amino butyric acid
GHT	Geniculohypothalamic tract
IGT	Intergeniculate leaflet
IHEC	Instituitional Human Ethics Committee
IPL	Interpeak Latency
kg	kilogram
mm	millimeter
ms	milliseconds
NPY	Neuropeptide Y
NREM	Non-Rapid Eye Movement
PACAP	Pituitary adenylate cyclase activating polypeptide
REM	Rapid Eye Movement
RHT	Retinohypothalamic tract
RLS	Restless Leg Syndrome
SCN	Suprachiasmatic Nucleus
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
SSS	Stanford Sleepiness Scale
SWC	Sleep wake cycle
VEP	Visual Evoked Potential
VIP	Vasoactive intestinal polypeptide

VISUAL EVOKED POTENTIALS (VEP)				
	Wave P_{100} Latency		Wave P_{100} Amplitude	
Sl no:	**Day shift personnel**	**Night shift personnel**	**Day shift personnel**	**Night shift personnel**
1.	96.5	97.1	8.77	8.7
2.	95.52	98	6.76	7.79
3.	95.1	97.5	8.87	7.68
4.	97.23	97.7	7.83	7.87
5.	96.49	98.65	7.78	8.32
6.	94.55	98.53	8.16	7.7
7.	93.7	97.24	8.88	7.78
8.	94.36	98.32	8.78	7.88
9.	94.1	98	8.81	6.77
10.	96.91	99.87	8.88	8.16
11.	94.6	99.56	8.78	7.6
12.	97.43	99.8	7.37	8.06
13.	95.98	99.87	8.31	9.3
14.	95.3	98.3	8.38	6.87
15.	94	98.6	8.88	8.27
16.	94.46	98.45	8.76	7.11
17.	94.61	99.4	6.86	7.7
18.	96.29	98.5	6.87	8.77
19.	95.43	98.88	7.83	8.8
20.	94.66	99	6	6.18
21.	95.68	97	6.63	7.21
22.	94.95	97.23	6.68	6.66
23.	93.7	97.89	6.87	8.87
24.	93.22	97.87	6.88	8.78
25.	93.21	99.88	6.37	7.87
26.	95.11	99.43	6.86	7.43
27.	97.15	99.56	8.66	6.7
28.	97.4	97.78	7.8	6.87
29.	96.23	99	7.77	6.71
30.	93.4	99.54	8.66	7.1
31.	93.22	98.45	8.66	8.8
32.	93	99.7	6.76	8
33.	93.85	99.5	8.66	7
34.	95.23	98.7	7.83	7.87

BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEP)										
Wave I Latency			**Wave II Latency**		**Wave III Latency**		**Wave IV Latency**		**Wave V Latency**	
Sl no:	**Day shift**	**Night shift**	**Day shift**	**Night shift**	**Day shift**	**Night shift**	**Day shift**	**Night shift**	**Day shift**	**Night shift**
1	1.8	1.79	2.79	2.77	3.5	3.69	4.52	4.62	5.4	6.01
2	1.7	1.77	2.5	2.89	3.5	3.54	5.06	4.98	5.49	6.15
3	1.61	1.74	2.76	2.78	3.59	3.69	5.2	4.94	5.35	6.3
4	1.67	1.71	2.74	2.85	3.55	3.67	5.08	5.2	5.42	5.89
5	1.78	1.77	2.72	2.76	3.57	3.63	5.08	5.79	5.4	6.35
6	1.77	1.75	2.76	2.78	3.65	3.67	5.23	5.76	6	5.95
7	1.68	1.8	2.79	2.89	3.56	3.68	4.87	5.65	5.11	6.02
8	1.67	1.78	2.8	2.82	3.94	3.66	4.98	5.88	5.69	5
9	1.68	1.48	2.89	2.78	3.57	3.72	4.94	5.73	5.49	6.01
10	1.62	1.79	2.76	2.86	3.59	3.67	5.08	5.68	5.3	6.22
11	1.6	1.34	2.8	2.85	3.59	3.72	5.08	5.75	5.44	5.99
12	1.62	1.78	2.75	2.81	3.57	3.74	5.06	5.08	6.19	5.93

13	1.44	1.45	2.78	2.79	3.68	3.68	4.99	5.95	5.4	5.56
14	1.71	1.75	2.75	2.86	3.7	3.69	4.77	5.81	5.6	5.93
15	1.64	1.47	2.81	2.85	3.55	3.68	4.73	5.8	5.44	5.4
16	1.7	1.79	2.86	2.76	3.56	3.69	5.08	5.73	5.31	6.21
17	1.7	1.73	2.74	2.88	3.51	3.67	5.08	5.95	5	5.99
18	1.62	1.74	2.74	2.87	3.56	3.65	5.08	5.56	6.21	5.89
19	1.78	1.38	2.71	2.86	3.7	3.74	4.5	5.77	5.34	5.78
20	1.65	1.75	2.89	2.87	3.51	3.71	4.81	5.88	5.48	5.56
21	1.68	1.73	2.8	2.78	3.55	3.67	4.77	5.85	5.48	5.21
22	1.67	1.76	2.7	2.87	3.67	3.68	5.52	5.02	5.52	5.11
23	1.66	1.47	2.88	2.88	3.59	3.66	5.95	5.06	5.69	5.23
24	1.75	1.72	2.86	2.86	3.7	3.7	5.83	5.06	5.23	5.2
25	1.68	1.74	2.89	2.79	3.59	3.71	5.77	4.52	5.59	6.1
26	1.68	1.74	2.88	2.99	3.72	3.74	5	5.06	5.44	6.4
27	1.85	1.75	2.75	2.83	3.94	3.67	5.85	5.2	5.21	5.97

28	1.68	1.49	2.87	2.84	3.69	3.6	5.02	5.08	5.4	6.02
29	1.47	1.39	2.8	2.52	3.65	3.66	5.06	5.08	5.23	5.73
30	1.6	1.7	2.88	2.65	3.66	3.74	5.06	4.52	5.59	5.87
31	1.68	1.77	2.75	2.66	3.65	3.69	4.52	4.87	5.4	5.88
32	1.49	1.75	2.79	2.91	3.9	3.74	5.06	4.98	5.03	6.15
33	1.66	1.78	2.78	2.9	3.66	3.69	4.87	4.94	5.35	6.12
34	1.66	1.71	2.76	2.87	3.65	3.7	5.08	5.08	5.02	6

BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEP)						
Sl no:	**Interpeak latency (I-III)**		**Interpeak latency (III-V)**		**Interpeak latency (I-V)**	
	Day shift	**Night shift**	**Day shift**	**Night shift**	**Day shift**	**Night shift**
1	1.96	2.35	1.54	2.32	3.79	4.32
2	1.98	2.08	2.23	2.25	3.88	3.99
3	1.99	1.71	1.75	2.4	4.5	3.23
4	1.48	2.04	1.63	2.34	3.81	3.77
5	1.93	2.35	2.21	1.9	4.65	4.15
6	1.95	1.35	1.88	2.35	3.82	3.5
7	1.75	2.35	2	2.4	3.86	4.27
8	2.48	2.52	1.96	1.99	4.04	4.54
9	1.99	1.65	2.22	2.36	3.78	4.45
10	1.98	2	2.04	1.97	3.79	4.81
11	1.82	1.69	1.77	1.96	3.21	4.98
12	1.8	2.33	2.42	1.8	4.75	4.15
13	1.99	2.52	2.34	2.12	3.98	4.52
14	1.96	2	1.99	1.95	4.19	3.79
15	2	1.58	1.85	2.35	3.77	3.97
16	1.75	1.71	1.87	2.36	3.84	4.3
17	1.95	1.96	2.33	2.37	3.83	4.27
18	1.65	2.4	2.69	2.22	4.21	4.23
19	1.98	2.44	2.67	2.31	4.02	3.96
20	1.75	2.48	1.99	2.22	4.04	4.02
21	1.96	2.08	2.38	2.31	4.25	3.89
22	1.98	2.1	1.85	1.97	3.88	3.99
23	1.75	2	1.75	2.11	3.69	4.15
24	1.95	2	1.58	1.98	3.82	4.33
25	2.4	1.35	1.96	1.85	4.04	3.96
26	1.93	1.35	1.92	1.88	3.71	3.97
27	1.95	1.94	2.69	2.34	4.21	3.9
28	1.9	1.94	2.04	2.08	3.88	4.25
29	1.94	1.56	1.99	2.17	3.63	3.98
30	1.88	1.9	2.13	2.21	4.08	4.06
31	1.96	2.35	2.34	2.3	3.9	3.87
32	1.96	2.08	1.38	2.25	3.89	3.99
33	1.97	1.71	1.75	2.98	4.23	3.98
34	1.48	2.04	1.63	2.35	3.81	4.3